

CARBON ALLOCATION AND FIELD RESIDUE DECOMPOSITION  
DYNAMICS OF MON863 BT CORN AND PUBLIC PERCEPTIONS OF  
TRANSGENIC CROPS IN CHINA

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Transgenic corn variety MON863, released in 2003, expresses the Cry3Bb protein from *Bacillus thuringiensis* (Bt), which has insecticidal activity against the corn rootworm (CRW). Despite the rapid adoption of Bt crops by farmers, public concern continues to mount over their potential environmental impacts. I assessed the effects of Bt corn and its parental NonBt hybrid on residue decomposition and carbon (C) allocation in field and greenhouse experiments. In the field, lignin concentration of Bt and NonBt corn residues, rates of residue decomposition, residue-colonizing decomposer communities and decomposition of the Cry3Bb protein were evaluated in litterbag studies. None of these variables were significantly different between the two hybrids. Differences observed were driven primarily by environmental factors related to time of sampling. The Cry3Bb protein decomposed nearly completely after 3.5 months in the field, indicating that the protein is unlikely to pose any significant ecological risks. Three greenhouse experiments were conducted to measure the allocation of labeled carbon ( $^{13}\text{C}$ ) in Bt and NonBt corn. There were no significant effects of Bt corn on  $^{13}\text{C}$  allocation, total C or lignin content of plant tissues. However, the lignin content of NonBt corn roots was significantly higher than that of Bt corn roots when plants were inoculated with CRW, indicating that induced systemic resistance, rather than presence of the Bt gene, may effect C budgets in agricultural

systems where CRW is present. NonBt corn was significantly taller than MON863 Bt corn. This was most likely the result of normal varietal variation as suggested by the differences in growth characteristics observed when Bt and NonBt corn from three maturity groups were compared.

A survey was conducted in China to explore the attitude(s) of Chinese consumers towards transgenic crops. Results showed that a large proportion of respondents held neutral positions towards or were unsure about transgenic crops. Respondents' attitudes shifted when provided information about the potential benefits and risks of transgenic crops. These results indicated that the commercial release of Bt rice is likely to encounter resistance from consumers. Future scenario testing indicated that increased public awareness would not necessarily improve consumer acceptance of transgenic rice.

## BIOGRAPHICAL SKETCH

Kai Xue was born on October 27<sup>nd</sup>, 1978, in Jiangsu, P.R. China. He earned his Bachelor's degree in Environmental Engineering from the Department of Chemical Engineering at Beijing University of Chemical Technology in May 2001, in China. In his thesis research, Kai isolated microorganisms that could endure the presence of phenol to degrade toluene. He earned his Master's degree in Environmental Science from the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China, in May 2004. In his Master's research, Kai studied microbial ecology using the phospholipid fatty acid analysis (PLFA) method. Mr. Xue started his Ph.D. program in the Field of Soil and Crop Sciences at Cornell University in August 2004 under the supervision of Prof. Dr. Janice Thies. Results of these studies are reported herein.

To my parents, and friends who support me all the time  
感谢我的父母和一直以来支持我的朋友

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## LIST OF ABBREVIATIONS

ADL, Acid Detergent Lignin;  
AMMI, Additive Main effects with Multiplicative Interactions;  
AR, Rotated out of Alfalfa;  
Bt, *Bacillus thuringiensis*;  
C, Carbon;  
CC, Continuously planted to corn;  
CLPP, Community-Level Physiological Profiling;  
CRW, Corn Root Worm;  
DGGE, Denaturing Gradient Gel Electrophoresis;  
EIQ, Environmental Impact Quotient;  
ELISA, Enzyme-Linked Immunosorbent Assay (ELISA)  
FDA, Food and Drug Administration;  
PLFA, Phospholipid Fatty Acid;  
GE, Genetic engineering;  
GEOs, Genetically Engineered Organisms;  
GMOs, Genetically Modified Organisms;  
IR, Insect Resistant;  
N, Nitrogen;  
SB, Shopping area in Beijing (survey respondents);  
SE, standard error;  
SG, Shopping area in Guanyun (survey respondents);  
TC, Transgenic Crops;  
T-RFLP, Terminal Restriction Fragment Length Polymorphism;  
MG, Maturity Group;  
UN, University or graduate school students (survey respondents).

# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 Challenges for Agriculture**

Despite the successes of the Green Revolution, small-holder farmers in many regions of the world, such as Africa, Asia and Latin America, still struggle with low agricultural productivity and food insecurity. Today, 854 million people, 14% of the world population, experience chronic and transitory hunger (Sanchez and Swaminathan, 2005). About half of these are in small-holder farming households. Increasing agricultural productivity, rather than putting more land into cultivation, is a key strategy for alleviating poverty and hunger. The situation will become increasingly dire as the world population continues to rise and agriculture has to feed 3.5 billion additional people over the next 50 years. This is a daunting job that requires dynamic agricultural development (Borlaug, 2007).

The Green Revolution resulted from the use of higher-yielding, fertilizer-responsive grain crop varieties that were produced by scientific plant breeding. These new varieties increased overall grain production successfully under the pressure of population growth (Conway and Toenniessen, 1999) and spared millions of hectares of land from being converted to agricultural use. However, increased use of fertilizers, pesticides and irrigation were also part of the Green Revolution. These inputs have had adverse environmental consequences and caused serious degradation of soil and water resources (Hails, 2002). The ceiling may have been reached for the Green Revolution to increase crop yields. One reason for the decline in the rate of yield increase is likely the environmental degradation caused by the Green Revolution itself (Conway and Toenniessen, 1999).

Global climate change is another current challenge for agriculture. Some changes,



such as increasing temperatures and decreasing precipitation over semiarid regions, may threaten global food security by decreasing the yields of primary crops in the near-term (Lobell *et al.*, 2008). The consequences of global climate change are also likely to include increasing drought, heat and/or cold, and water-logging in agricultural ecosystems (Borlaug, 2007).

Overall, agriculture faces the challenges of needing to be more productive, conserving natural resources, and protecting the environment as well. Transgenic crop technology is one possible option for addressing these multi-faceted challenges.

## **1.2 Introduction of Transgenic Crops**

Genetic engineering (GE) has been hailed as a promising new approach to plant breeding, but it is highly controversial as well. The small- and large-scale ecological consequences of cultivating transgenic crops (TCs), created by GE techniques, are of major concern to the public.

In order to obtain particular desirable traits, genes from one organism are transferred deliberately into another organism's genome by GE, creating transgenic organisms (Kleter *et al.*, 2007). Transgenes are composed minimally of a target gene sequence associated with the desired traits, a promoter and other elements. All these elements may come from different organisms (Snow *et al.*, 2005). Transgenic organisms are also referred to as genetically engineered organisms (GEOs), genetically manipulated organisms (GMOs) and bioengineered organisms. In contrast to transgenic organisms, cisgenic organisms contain a gene transferred from a different location within the same genome (Kleter *et al.*, 2007).

The practice of manipulating the genetic background of crop plants is not new. Since the beginning of agriculture, crops and domesticated animals have been

“genetically modified” by selective breeding methods employing sexual reproduction. These approaches include: “1) assembling or generating new genetic diversity, 2) selecting and testing different genotypes to identify superior varieties, and 3) the release, distribution, and commercialization of new progeny” (Snow *et al.*, 2005). TCs are distinguished from traditional varieties only insofar as GE, rather than traditional approaches, has been used to generate new genetic diversity. With traditional methods, DNA from the chosen parents is mixed and sorted randomly and can be exchanged only within the same species. GE overcomes these limitations of traditional methods, which are slower and considered less accurate (Ervin *et al.*, 2003). GE allows for the transfer of specific genes, which code for certain desirable traits, beyond the boundaries of species and is less time-consuming (Fernandez-Cornejo and McBride, 2002). The plants created by use of GE technologies contain genes for traits that cannot be obtained by traditional breeding methods (Snow *et al.*, 2005).

TCs have become the most rapidly adopted crop technology in recent human history (James, 2006). In 1994, the Flavr-Savr® tomato was approved as the first commercial TC released by the US Food and Drug Administration (FDA) (Shehata, 2005). Since the first large-scale commercial planting in 1996, the global area planted with TCs has increased steadily at a double-digit rate for several consecutive years. In 2007, the area planted with TCs reached a total of 114.3 million ha, 67-fold more than in 1996 (James, 2007). Based on the sale price of TP seed, plus any technology fees that apply, the global market value of TCs was estimated by Cropnosis to be US\$ 6.9 billion in 2007, and predicted to be over US\$ 7.5 billion in 2008 (James, 2007).

The cumulative number of farmers who planted TCs was 55 million in 2007. TCs were adopted rapidly by farmers in industrialized countries, and then spread to some developing countries. The proportion of TCs planted in developing countries has continued to increase from 14% in 1997 to 43% of the global area planted to TCs in

2007 (James, 2000, 2007). Among the 23 countries planting TCs, the USA, Argentina, Brazil, Canada, India and China are the principal adopters. In 2007, the area planted to TCs in the USA reached 57.7 million ha, 50% of the global area planted to TCs.

In 2007, soybeans, corn, cotton and canola, the four main TCs, accounted for 51%, 31%, 13% and 5% of the global area planted to TCs, respectively (James, 2007). The main TC traits in use are herbicide tolerance (HT) and insect resistance (IR), accounting for 63% and 18% of global TC plantings in 2007, respectively (James, 2007). Recently, stacked products that can contain more than one trait and confer multiple benefits in a single transgenic variety have been increasingly deployed. In the USA, 63% of transgenic corn, 78% of transgenic cotton, and 37% of all TCs were stacked products in 2007 (James, 2007). Crops have also been modified to tolerate environmental stress, resist diseases, increase nutritive value and shelf life, grow with less nitrogen (N) fertilizer, produce pharmaceuticals, biodiesel and industrially useful materials, modify their lignin content, aid in food processing or, accumulate environmental toxins.

### **1.3 Benefits of Transgenic Crops**

All agricultural practices can have effects on associated natural systems and the economic viability of agricultural production systems (Hails, 2002). James (2007) asserts that TCs provide some environmental, economic, health and social benefits and contribute to a more sustainable agriculture.

GE technologies can enhance agricultural productivity greatly. For example, IR TCs have the potential to reduce crop losses by protecting crops from pests without the need for insecticides, which are relatively expensive for farmers in developing countries and pose health risks. Use of IR TCs reduces pest feeding, which in turn may reduce secondary infections by plant pathogens. For example, Munkvold *et al.* (1997,

1999) observed that mycotoxin levels were reduced in the tissues of Bt plants. These mycotoxins are commonly released by fungal pathogens and may be harmful to humans and livestock (Munkvold *et al.*, 1997 and 1999). Use of TCs that are resistant to common soil stresses, such as drought, salinity and acidity, may help to increase production on marginal or degraded agricultural land, and hence, increase production in areas where traditional crop varieties fail. Since many areas in developing countries have poor soils, TCs may play a vital role in alleviating poverty, malnutrition and hunger (James, 2004). They could also be regarded as part of the solutions for addressing the challenges of global climate change.

Specifically, IR crops can help to reduce the conventional practice of spraying pesticides on crops, which has serious detrimental effects on human health and the environment (Ando and Khanna, 2000). Although pesticides can increase crop yield by controlling insects, diseases or weeds in the field, they destroy ecological diversity on agricultural lands by killing most insects, of which only a few are target organisms. Moreover, agrichemicals affect human health by entering the food supply and polluting water resources (Ando and Khanna, 2000). Use of TCs can help to reduce the global use of pesticides and other agrichemicals and thus benefit the environment, economy and farmers' welfare. Specifically, crops that are engineered to express the Cry protein gene from the soil bacterium *Bacillus thuringiensis* (Bt crops) are designed to target only certain groups of pests and are thus more selective than broad-spectrum synthetic insecticides. In agricultural ecosystems planted to Bt crops, pesticide use can be reduced and beneficial insects and natural enemies of pests may have a greater chance of survival (Kleter *et al.*, 2007). Brookes and Barfoot (2006) estimated the cumulative reduction in pesticides resulting from growing IR crops in the world was 289,000 metric tons of active ingredient (a.i.) from 1996 to 2006. Based on the Environmental Impact Quotient (EIQ) developed by Kovach *et al.* (1992 and

updated annually), the associated environmental impact of pesticide use on areas planted to IR crops decreased 15.5% during that period. A survey conducted by Huang *et al.* (2005) in 2002 and 2003 in China showed that full adopters of insect-resistant transgenic rice did not suffer adverse health effects, while 3 to 10.9% of households who planted IR rice and non-IR rice together or only non-IR rice were reported to be adversely affected by pesticide use. However, the benefit of TCs to decrease pesticide applications may depend on the pest species targeted. For example, although expression of the Cry1Ab gene in corn protected plants from *O. nubilalis* (European corn borer, ECB) and reduced the amount of pesticide needed to control it, these insects are only part of the overall pest complex affecting field grown corn. Thus, the insecticide use in most of the corn-growing areas in the Midwest U.S. may not be reduced substantially because only a percentage of the insecticides used were for ECB control, even before the introduction of Bt corn. This case is different from transgenic potatoes and cotton, where the dominant pests are controlled by the presence of the Cry protein (Obrycki *et al.*, 2001).

Large-scale adoption of glyphosate-tolerant soybeans in the U.S. has led to an increase in glyphosate use (Wolfenbarger and Phifer, 2000; Carpenter *et al.*, 2002) and decreases in the use other major herbicides (Carpenter *et al.*, 2002). Compared to many other herbicides, glyphosate is less environmentally persistent, with low or no toxicity to vertebrates and invertebrates (Snow *et al.*, 2005). The use of HT crops may encourage the use of reduced or no-tillage practices, which can preserve soil quality and contribute to carbon (C) sequestration (Snow *et al.*, 2005).

The potential benefits of TCs still being developed include the “improvement in fruit and vegetable shelf-life and organoleptic quality, improved nutritional quality and health benefits in foods, improved protein and carbohydrate content of foods, and improved fat quality” (Uzogara, 2000). In addition, TCs may be employed as bio-

factories to produce some raw materials for industrial uses without attendant environmental problems that current factories have, such as the disposal of waste gas, water or solids.

#### **1.4 Potential Risks of Transgenic Crops**

Despite the rapid adoption by farmers of TCs, more and more concerns have been raised by the public about their potential environmental and health effects. In the controversy surrounding this issue, public opinion has been highly polarized (Ando and Khanna, 2000). Beside the overarching moral debates, the biggest obstacle for public acceptance of TCs is uncertainty with regard to their environmental impacts. The main concerns are their “unintended” and non-target effects, potential effects on human health, the outcomes of gene flow, and loss of effectiveness.

Two different regulatory procedures, product-related standards and process-related standards, are employed in different countries to administer the commercial use of TCs. The assumption behind product-related standards, adopted in the U.S., is that “the GE technique has no essential difference with traditional biotechnology, so the safety assessment should be aimed at the final products”. This is referred to as “substantial equivalence”. The assumption behind process-related standards, adopted in Europe, is that the “genetic engineering technique itself has a certain potential hazard, so the safety of the procedure should be the crucial part of assessment” (Deng *et al.*, 2008).

The methodological components of risk assessment include hazard identification, exposure assessment, assessment of potential consequences, risk characterization and mitigation options (Hill, 2005). The basic principles underlying risk assessment of TCs include the substantial equivalence principle (OECD, 1993) and case-by-case assessment (FAO/WHO, 2000).

### 1.4.1 Unintended Effects

Unintended effects may occur during the process of creating TCs by GE technologies. Currently, it is still technologically difficult to intentionally insert transgenes into a recipient organism's genome at a fixed-point (Deng *et al.*, 2008). Transgenes are often inserted into random chromosomal locations and could be at multiple sites in the genome. This may cause positional effects and lead to unintended phenotypes. The level and consistency of gene expression can be affected by the location in which transgenes are integrated. It is possible for inserted transgenes to interrupt native genes or their promoters, cause small-scale rearrangements (Windels *et al.*, 2001), have unanticipated interactions between transgenes and native genes, increase mutations, and affect multiple traits (pleiotropic effects). Usually, it is difficult to figure out the reasons for unintended effects or to even detect some small unintended effects, which “may depend on cumulative action, specific environmental conditions, or introgression into different genetic backgrounds” (Snow *et al.*, 2005). However, unintended effects are not unique to TCs as traditional breeding methods may also cause unintended effects.

Altered lignin concentrations and slower rates of residue decomposition are concerns that have been highlighted most recently. Change in the rate of plant residue decomposition in the field is one of the major concerns in view of the effects this may have on greenhouse gas emissions and C sequestration in soils. Most studies on the unintended effects of Bt crops focus on residue decomposition rate (Table 1.1) and lignin concentration (Table 1.2). Saxena and Stotzky (2001b) found that the stems of Cry1Ab Bt corn (Bt11, MON810 and Bt176), growing in a plant growth room or in the field, had a significantly higher (33–97% higher) lignin concentration than that of

Table 1.1 Summary of the effects of Bt crops on decomposition rate.

Reference	Materials	Protein	Location	Measurements	Results
Hopkins and Gregorich, 2003	Corn leaves	Cry1Ab	Lab	Soil respiration	No effect
Castaldini <i>et al.</i> , 2005	Corn leaves and stems	Cry1Ab	Lab	Soil respiration	Lower in Bt
Flores <i>et al.</i> , 2005	Corn leaves and stems; biomass of rice, tobacco, canola, cotton, and potato	Cry1Ab corn/rice; Cry3A potato; Cry1Ac cotton/canola/tobacco	Lab	Soil respiration	Lower in Bt
Cortet <i>et al.</i> , 2006	Wheat straw in Bt/NonBt corn field	Cry1Ab	Field	Litterbag	No effect
Fang <i>et al.</i> , 2007	Pooled corn residues	Cry1Ab	Lab	Soil respiration	No effect
Zwahlen <i>et al.</i> , 2007	Corn leaves	Cry1Ab	Field	Litterbag	No effect
Lehman <i>et al.</i> , 2008	Pooled corn stalks + leaves	Cry1Ab, Cry3Bb, Cry1Ab +Cry3Bb	Field	Litterbag	No effect
Tarkalson <i>et al.</i> , 2008	Corn cobs, stalks, leaves	Cry1Ab	Field	Litterbag	No effect



Table 1.2 Summary of the effects of Bt crops on lignin concentration.

Reference	Materials	Protein	Location	Measurements	Results
Escher <i>et al.</i> , 2000	Corn leaves	Cry1Ab	Lab	Concentration (% of biomass) by ADL	Lower in Bt
Saxena and Stotzky, 2001b	Corn stems	Cry1Ab	Plant growth room & field	Concentration (% of biomass) by fluorescence microscopy, staining with toluidine blue, AcBr.	Higher in Bt
Jung and Sheaffer, 2004	Whole corn plant	Cry1Ab	Field	Concentration ( $\text{g kg}^{-1}$ ) by ADL; AcBr; Klason	No effect
Flores <i>et al.</i> , 2005	Corn leaves and stems; and other plants	Cry1Ab corn/rice, Cry3A potato, Cry1Ac cotton/canola/tobacco	Lab	Concentration (% of biomass) by AcBr	Higher only in Bt corn
Poerschmann <i>et al.</i> , 2005	Corn stems, leaves	Cry1Ab	Field	Concentration (% of biomass) by GC-MS	Higher only in Bt stems
Fang <i>et al.</i> , 2007	Corn roots, stems, leaves	Cry1Ab	Field	Concentration (% of biomass) by ADL	Higher in Bt (1 of 5 pairs)
Lehman <i>et al.</i> , 2008	Pooled corn stalks + leaves	Cry1Ab, Cry3Bb, Cry1Ab +Cry3Bb	Field	Concentration ( $\text{g kg}^{-1}$ ) by ADL	No effect
Tarkalson <i>et al.</i> , 2008	Corn cobs, stalks, leaves	Cry1Ab	Field	Concentration (% of biomass) by ADL	No effect

their respective non-transgenic isolines, which they suggested may account for the slower decomposition rates they observed for Bt corn residues. Other studies confirmed that Bt Cry1Ab corn had a slower decomposition rate (Castaldini *et al.*, 2005) or higher lignin concentration (Fang *et al.*, 2007) or both (Flores *et al.*, 2005). However, conflicting results were obtained in other studies. A lower lignin concentration was found in Cry1Ab Bt corn (X4334-EPR, Novartis, previously Northrup King) between 2 and 4 weeks after putting water soaked leaves in plastic boxes without soil (Escher *et al.*, 2000). However, as a soil-free system was used, this study can be criticized because it does not represent the natural process of decomposition. In a laboratory incubation study, Hopkins and Gregorich (2003) found there was no detectable difference in the decomposition of Bt corn (Pioneer 38W36) and NonBt (Pioneer 3893) corn leaves as measured by the release of CO<sub>2</sub> during microbial respiration. Poerschmann *et al.* (2005) studied the molecular composition of Cry1Ab Bt corn and found the total lignin concentration was higher for stems of transgenic lines compared to near-isolines, but there was no significant difference in the lignin concentration of leaves between transgenic lines and near-isolines. Jung and Sheaffer (2004) reported that the ability to express the Cry1Ab protein gene did not alter the lignin concentration in commercial maize hybrids growing in the field. They chopped up all parts of the plant and then analyzed the whole plant samples. In Europe in 2006, Cortet *et al.* studied the decomposition of wheat straw to assess the effects of growing Cry1Ab Bt and NonBt corn in three different climate zones and found no negative effects of the Bt corn on decomposition processes, which were mainly affected by seasonal weather conditions in their study. Zwahlen *et al.* (2007) used litterbags with three different mesh sizes and found no major changes in the decomposition of Cry1Ab Bt maize residue as compared to its non-transgenic counterpart. Tarkalson *et al.* (2008) used litterbags in the field and did not detect any

difference in the rates of decomposition, mass of C remaining over time or lignin concentration between two Cry1Ab hybrids and their NonBt isolines. Lehman *et al.* (2008) placed litterbags containing mixed stalks and leaves in the field for 22 months and found no differences in decomposition rates or lignin concentrations of the residues between Cry1Ab, Cry3Bb, or a stacked Cry1Ab and Cry3Bb Bt hybrid and one hybrid representing the base genetics of the transgenic hybrids. It is important to point out that most of the literature cited above used the term “lignin content”, but they actually measured the lignin concentration (lignin/biomass, % or g kg<sup>-1</sup>).

Griffiths *et al.* (2007) found Bt maize had a significantly higher shoot C:N ratio than its NonBt counterparts for two of eight paired lines of Cry1Ab Bt maize and the near-isogenic NonBt maize they measured. A slightly lower C:N ratio was observed by Escher *et al.* (2000) in leaves of Cry1Ab Bt corn, as well as a higher content of soluble carbohydrates. A higher C:N ratio may cause slower residue decomposition as N is commonly limited for decomposers (Swift *et al.*, 1979). Soluble carbohydrates are labile fractions of residues for decomposition (Berg, 1986).

#### **1.4.2 Non-target Effects**

To study the effects of TCs on non-target organisms, biological, physical and geographical factors should be considered, especially in relation to the spatial and temporal variability of these factors (Snow *et al.*, 2005). Any effects observed could be related to direct exposure to products of the transgenes involved or be indirectly caused by changes in the physical or biological environment and/or changes in agricultural practices resulting from the use of TCs (Hails, 2000). Insect-resistant TCs, especially Bt crops, have received the greatest attention for their non-target effects (Snow *et al.*, 2005).

#### 1.4.2.1 Exposure to Bt Protein

Soil organisms may be affected by TCs through exposure to the Bt protein. The possible routes for Bt protein to enter into soils include root exudates (Saxena *et al.*, 1999), plant residues (Tapp and Stotzky, 1998) and pollen (Losey *et al.*, 1999).

Different crops were observed to affect the release of Bt protein in root exudates (Saxena *et al.*, 2004). The exudates of Bt corn, potato and rice contained Bt protein, but that of Bt cotton, canola and tobacco did not. However, for 12 different Cry1Ab corn hybrids, the Bt protein was detected in root exudates of all hybrids (Saxena *et al.*, 2002). Saxena *et al.* (1999) found that Cry1Ab Bt protein released in corn root exudates could bind to surface-active particles (clays and humic acids) in soil and both retard its biodegradation and increase its persistence in soil. Saxena and Stotzky (2001a) found that the bound proteins from Bt corn could be accessed for use as an N source by microbes only in the presence of another N source, but that the bound protein could not be used as a C source at all. The anti-lepidopteran toxin (Cry1Ab protein) from corn root exudates was detected in soil (52% sand, 36% silt, and 12% clay) for 180 days (Saxena *et al.*, 2002). However, in a study by Head *et al.* (2002), no detectable Cry1Ac protein was found in soil samples (no information on soil type) collected 3 months after the last season's tillage from within and outside six fields planted with Bt cotton, although the residues had been incorporated into the soil for 3-6 consecutive years. No Cry3Bb1 protein was detected by use of the enzyme-linked immunosorbent assay (ELISA) method in soil planted with Bt corn for one or three consecutive years near Manhattan, KS (Ahmad *et al.*, 2005). However, in another location near Scandia, KS, with one-year of Bt corn planting history, Cry3Bb1 protein was detected at a level of 3.38-6.89 ng g<sup>-1</sup> dry soil during the growing season in soil samples collected from areas near the base of the plants, while no protein was detected

in soil samples collected between rows. The protein extraction efficiency was measured by adding a known quantity of purified protein to both soils. The extraction efficiency was lower for the Manhattan than for Scandia soil. This could be explained by differences in the clay content of the Manhattan (36%) and Scandia soils (5%). All these results indicate that both the protein structure and soil type affect the persistence of Bt proteins in soil.

Bt protein can be released into soil from decaying plant residues left in the field after harvest. Hopkins and Gregorich (2005) found a rapid decomposition rate of the  $\delta$ -endotoxin from post-harvest Cry1Ab corn residues in a laboratory study. No protein was detected after incubation for 14 days in soil (39% sand, 27% silt, and 34% clay). Zwahlen *et al.* (2003) found a rapid initial decay followed by a slow degradation of Cry1Ab protein in transgenic corn leaves contained in litterbags buried in fields with or without tillage management. In the tilled field, there was a lag time, 30–60 days, before the first rapid decline in detectable protein was observed and there was no further degradation of the protein during winter. Bt protein could still be detected at the end of the experiment after 200 and 240 days, although the amount of plant residue remaining in the litterbags was quite small at that time. The soil used in their study was Cambisol loam (50% sand, 33% silt and 17% clay).

Even though Bt protein adsorbs and binds to surface-active particles, it may still retain its insecticidal activity. After adding purified Cry1Ab protein to non-sterile soils and incubating for 234 days, larvicidal activity could still be detected on tobacco hornworm larvae (Tapp and Stotzky, 1998). These results indicate that Bt protein may accumulate in soil and hence, increase the potential for long-term (cumulative) effects on the soil biota.

#### 1.4.2.2 Direct Effects

The first recorded non-lepidopteran, non-target, beneficial insect affected by Cry1Ab (Hilbeck *et al.*, 1998a, b) and Cry2A (Hilbeck *et al.*, 1999) proteins from Bt corn was the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), an important polyphagous predator in many crops (Dutton *et al.*, 2002). For direct effects of Bt protein, they found a significantly higher mortality of immature green lacewing larvae fed on an artificial diet with Cry1Ab protein incorporated. However, conflicting results were obtained by Romeis *et al.* (2004) who found that the Cry1Ab protein did not have a direct effect on the larvae. The indirect effect they observed is discussed in section 1.4.2.3 (Indirect Effects).

In laboratory incubations, Saxena and Stotzky (2001a) found that Cry1Ab Bt protein in root exudates and residues of Bt corn had no apparent effects on earthworms, nematodes, protozoa, bacteria, or fungi in soil. The percent mortality and weight of earthworms after 40 days in soil planted with Bt corn and after 45 days in soil amended with Bt corn residues were not significantly different from their NonBt controls. The number of protozoa and nematodes and colony-forming units of culturable bacteria (including actinomycetes) and fungi were also not significantly different between rhizosphere soil of Bt and NonBt corn or between soil amended with residues of Bt and NonBt corn. However, it must be pointed out that culturable microorganisms studied here represent only a fraction of the total number of microorganisms found in soil. In a field experiment conducted over two years in NY, Devare *et al.* (2004) used terminal restriction fragment length polymorphism (T-RFLP) analysis, a DNA fingerprinting method that captures both the culturable and unculturable fractions of the microbial community, to study the effect of Bt corn (MON863) expressing the Cry3Bb protein on soil microbial community composition. No differences between the transgenic and non-transgenic isolines in the bacterial

community composition of their respective rhizosphere soils were detected in these DNA fingerprints. In addition, no differences in soil microbial biomass or activity (as measured by N mineralization potential, short-term nitrification rate, and soil respiration) were detected between the transgenic and non-transgenic isolines. The application of tefluthrin, a pyrethroid insecticide used commonly to control insect pests of corn, to the NonBt isolate used in this study was also reported to have no adverse effects on any microbial variable measured, except for a decreased respiration in soils planted to Bt corn in one of the two years (Devare *et al.*, 2004). Whether decreased respiration represents an adverse effect or not depends on system-level goals. Slower turnover of residues may slow nutrient release, but it also slows C lost as CO<sub>2</sub> to the atmosphere and thus C is retained in the soil for a longer period. Overall, the results reported by Devare *et al.* (2004) indicate that growing Bt corn at this NY field site had no adverse effects on the soil microbiota or their activities, based on the variables measured. The same conclusion was drawn by Devare *et al.* (2007) for MON863 (Cry3Bb) corn grown for three consecutive years in NY where no significant effects on soil microbial biomass, N mineralization potential, short-term nitrification rate, or respiration rate were detected.

Castaldini *et al.* (2005) reported differences in rhizosphere bacterial communities associated with three corn lines, two Cry1Ab Bt corn hybrids (Bt11, Bt176) and one NonBt hybrid (NK4640, the parental line of Bt11), detected by denaturing gradient gel electrophoresis (DGGE) analysis. A significantly lower level of mycorrhizal colonization in Bt176 corn roots was also observed in microcosm experiments. In their experiments, differences in rhizosphere bacterial abundance (both total and active bacteria), culturable rhizosphere heterotrophic bacteria and mycorrhizal colonization were observed between Bt and NonBt hybrids. Moreover, soil respiration, mycorrhizal colonization by indigenous endophytes and soil bacterial communities were also

affected by adding Bt residues to soil for up to 4 months, compared to soil with NonBt residues added. However, only one NonBt hybrid, the parental line of Bt11, was included in this study. With no NonBt parental line for Bt176 to compare to, it is not possible to discern whether any observed differences were due to the insertion of Bt gene or inherent differences between hybrids and/or maturity groups.

The substrate utilization profiles (Biolog, Inc., Hayward, CA) of soils amended with Cry1Ab Bt residues were different from those of soils amended with NonBt residues in both the laboratory and field studies reported by Fang *et al.* (2007). However, their DGGE results revealed only slight or no differences in bacterial community composition between the Bt and NonBt treatments. Baumgarte and Tebbe (2005) investigated rhizosphere bacterial community composition using the single-strand conformation polymorphism (SSCP) method and found the effect of Cry1Ab corn was less than that of other environmental factors, such as plant age or field heterogeneities. Similar results were obtained by use of the community-level physiological-profiling (CLPP, Biolog, Inc.) and phospholipid fatty acid analysis (PLFA) methods in both a field trial (Griffiths *et al.*, 2005) and a greenhouse experiment (Griffiths *et al.*, 2006). Blackwood and Buyer (2004) studied both Cry1Ab and Cry1F Bt corn planted in three different soils in a growth chamber experiment. Differences in the rhizosphere bacterial CLPP profiles between Bt and NonBt corn and between the different hybrid pairs were found only for the clay soil though “the amount of variability accounted for was small”, while no differences were found using the PLFA method in their study. Overall, Blackwood and Buyer still concluded that the effect of Bt corn on soil microbial communities was small. Icoz *et al.* (2008) used three hybrids of Cry1Ab corn, one Cry3Bb corn hybrid and their respective NonBt counterparts to evaluate the effects of the Bt proteins on microbial diversity, which was assessed by dilution plating, DGGE, and the activity of enzymes catalyzing



decomposition reactions (arylsulfatases, acid and alkaline phosphatases, dehydrogenases and proteases). After four consecutive years, no significant effects on any of these variables resulting from growing the Bt crop were found. It is interesting to note that differences in soil microbial communities between Bt and NonBt corn treatments were only detected in the physiological tests (Biolog/CLPP) used in these studies.

A significant quantity of Bt protein can be expressed in Bt maize pollen (Fearing *et al.*, 1997), which can be dispersed by wind and become dangerous to some non-target organisms that ingest it. A laboratory study conducted by Losey *et al.* (1999) at Cornell University showed higher mortality and slower rates of growth of monarch butterfly larvae eating milkweed leaves dusted with pollen from N4640-Bt corn than those of larvae eating leaves dusted with untransformed corn pollen or leaves without pollen. A field study also revealed a significant mortality of monarch butterfly [*Danaus plexippus* L. (Lepidoptera: Danaidae)] larvae caused by pollen of Bt corn that was deposited naturally on milkweed in the field. However, no sub-lethal effects were found on adults from larvae surviving after a 48-h exposure to Bt pollen (Hansen-Jesse and Obrycki, 2000). Yet, a two-year collaborative research project between scientists in several U.S. states and in Canada did not show the same results. Sears *et al.* (2001) found Bt expression in pollen was low in most commercial hybrids and its impact on monarch butterfly populations was negligible.

In spring 2007, a mysterious phenomenon affecting honey bees called "Colony Collapse Disorder" (CCD) was observed in the U.S. and some other countries, such as Germany. Reported in the *New York Times* (2007), the damage to U.S. agriculture will be enormous as numerous crops rely on bees for pollination (Barrionuevo, 2007). The planting of TCs, especially Bt crops, was suggested to be one factor contributing to the CCD phenomenon. However, a study conducted at the University of Jena from 2001

to 2004 showed no evidence of chronic toxic effects of Bt176 or Mon810 Bt Cry1Ab corn on bee populations ([www.gmo-safety.eu/en/safety\\_science/68.docu.html](http://www.gmo-safety.eu/en/safety_science/68.docu.html)). Cox-Foster *et al.* (2007) found Israeli acute paralysis virus of bees was strongly correlated with CCD by using the metagenomic approach to survey microflora in CCD hives, normal hives, and imported royal jelly for 3 years.

#### **1.4.2.3 Indirect Effects**

Through trophic interactions, Bt plants may pose an indirect hazard to higher trophic level, non-target organisms, such as arthropod predators and parasitoids (Hilbeck *et al.*, 1998 a,b, 1999) that consume herbivorous insects, whether the herbivore is itself susceptible or not. It is not surprising that susceptible organisms and their natural enemies, especially specialist natural enemies, could be affected by Bt crops. If predator insects have receptors for the specific Bt protein expressed and consume insects feeding on tissues containing this protein, they could be affected. The risk to these non-target insects in this case is related to the likelihood and level of exposure to the protein, which will be affected by the ability of the prey to metabolize and deactivate the protein. Head *et al.* (2001) studied the secondary exposure risk of predators and parasitoids to Cry1Ab Bt corn by measuring Bt protein levels in different phytophagous insects after they had eaten corn tissues containing the Cry1Ab protein or artificial diets containing different levels of purified Cry1Ab protein. Their results demonstrated that the levels of Bt protein in insects feeding on Bt residues and artificial diets containing Cry1Ab protein were many-fold less than their original levels, respectively, but varied with insect species and food source. In another study, the presence of Cry1Ab protein was detected in the integument and casts of earthworms after they had fed for 40 days in soil planted with Bt corn and after feeding for 45 days in soil amended with Bt corn residues. However, the Bt protein

was not detected in the integument or casts after the earthworms were placed in fresh soil for 2-3 days (Saxena and Stotzky, 2001a).

The effect of Bt protein exposure on non-target predators was studied by Hilbeck *et al.* (1998a, 1999) in a laboratory feeding experiment. They found that the mean total mortality for immature *C. carnea* larvae reared on Bt-fed (Cry1Ab and the protoxins of Cry1Ab and Cry2A) *Spodoptera littoralis* (Boisduval) (lepidopteran, non-target pest used) was significantly higher than in the control. However, Dutton *et al.* (2002) reported that chrysopid larvae were not affected by being reared on spider mites, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), fed on Cry1Ab corn. Romeis *et al.* (2004), who found that Cry1Ab Bt protein did not have any direct effects on the larvae, suggested that the effects of Bt corn observed by Hilbeck *et al.* (1998a, 1999) were “prey-quality mediated rather than direct toxic effects”.

To assess the effects of Cry1Ab protein on the insect parasitoid *Campoletis sonorensis* (a solitary endoparasitoid of noctuid larvae), Sanders *et al.* (2007) conducted a laboratory study. They found that, although the adult parasitoid contained no detectable Cry1Ab protein, the biomass of adult parasitoids reared on Bt maize-fed *Spodoptera frugiperda* larvae was significantly lower (15-30%) than that of parasitoids reared in hosts fed NonBt corn tissue. However, Walker *et al.* (2007) found the effect of reduced nutritive quality (1/8<sup>th</sup> or 1/6<sup>th</sup> of standard amounts of key ingredients) of pests feeding on Bt potato was greater than that of Cry1Ac protein on the survival of parasitoids (*Hymenoptera: Braconidae*) on non-target lepidopteran pests of Bt potato, *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae). A hyperparasitoid is a secondary parasitoid that parasitizes the primary parasitoids. A study conducted by Pruetz *et al.* (2004) showed that the hyperparasitoids *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae), mediated through the herbivore *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and its

primary parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), was affected indirectly by Cry1Ab Bt corn. Females of *T. howardi*, the facultative hyperparasitoid that was tested, put on less weight and had fewer offspring in the Bt-group than in the control.

Escher *et al.* (2000) studied the microorganisms colonizing the feces of the woodlouse *Porcellio scaber* (Crustacea: Isopoda) fed on Cry1Ab Bt corn and NonBt corn. They reported a higher number of bacteria on the feces of *P. scaber* fed on non-transgenic corn than on the feces of the transgenic-fed woodlice. However, no difference was found for bacterial growth on corn leaves or fungal growth on feces.

A cascade of ecological changes may occur if TCs affect one species or a certain group of species. However, the effects could also be negligible at the community level when there is a high redundancy of ecological functions (Snow *et al.*, 2005). The effects of Bt proteins on predators and parasitoids may cause a change in the whole arthropod community. By use of suction sampling methods, the canopy invertebrate communities in fields of conventional cotton sprayed with appropriate insecticides, unsprayed conventional cotton, and unsprayed Bt cotton (Cry1Ac, stacked Cry1Ac + Cry2Aa) were compared over three years (Whitehouse *et al.*, 2005). The results showed that Bt cotton did not have significant effects on key species in the arthropod community compared to conventional cotton. The practice of spraying insecticide contributed most to differences observed between the two communities, although a slight difference between unsprayed conventional and unsprayed Bt cotton was also identified. The subtle shift caused by Bt cotton might be driven by a reduction in *Helicoverpa* and other lepidopteran populations, the targets of Cry1Ac and Cry2Aa. The authors suggested that the long-term effects of transgenic cotton on these communities should be monitored. Excluding the pest targeted by Cry1Ac Bt cotton, the abundance and diversity of all other arthropods were compared by Sisterson *et al.*

(2004) in field plots of Bt cotton, NonBt cotton and a mixture of Bt and NonBt cotton at two sites. The effects of site, plant height, and cotton type on arthropod abundance were significant, but the effects of year and block were not. The abundance in Bt plots was significantly lower than in mixed plots and marginally significantly lower than in NonBt plots. Within the mixed plots, no difference in arthropod abundance was found between the Bt and NonBt plants. Finally, Sisterson *et al.* (2004) concluded that arthropod abundance and diversity depended on many factors and the effect of Bt crop was only a minor one. Ahmad *et al.* (2005) worked with Cry3Bb1 Bt and NonBt corn and found the abundance of surface and below-ground, non-target arthropods in these fields were not significantly different.

Changes in agricultural land use caused by introducing TCs may have indirect negative effects on environmental biodiversity. The HT TCs have the ability to tolerate herbicide and make the removal of weed species more efficient, but at the same time may result in reducing the abundance and diversity of weeds and all species associated with them, including beneficial species that rely on weeds as their primary food source. Similarly, parasitoids cannot avoid indirect toxicity of IR TCs (Hails, 2000). With the reduced pest populations caused by TCs, predators that may be beneficial to the environment and species related to them in the food web may also be affected due to decreases in their primary food resources.

In order to assess the effects of TCs on farmland wildlife (including birds), a field study was conducted by the UK government between 2000 and 2002. Genetically modified, HT sugar beet, fodder beet, spring oilseed rape and fodder maize were used in this three-year study, each planted at twenty or more sites. The data were analyzed and published in a series of eight papers in the *Philosophical Transactions of the Royal Society of London* (Brooks *et al.*, 2003; Champion *et al.*, 2003; Hawes *et al.*, 2003; Haughton *et al.*, 2003; Heard *et al.*, 2003a, b; Roy *et al.*, 2003; Squire *et al.*,

2003). The results revealed that weed biomass and seed rain in the plots planted with HT TCs were less than a third that of the conventional plots for sugar beet and oilseed rape. This resulted in reduced food availability for mammals and birds. However, in transgenic maize plots, there was almost twice as much weed biomass and seed rain compared with conventional maize. This was attributed to the fact that atrazine, the herbicide used on the conventional maize, was more effective in controlling weeds than the glufosinate used on the transgenic maize.

TCs have a narrow spectrum of effectiveness on certain pests. Using household survey data in which farmers were asked to recall their pesticide use, Wang *et al.* (2006) found that pesticide use increased to control the secondary pest populations for cotton, mainly mirids, after seven years of planting Bt cotton, which controls only the bollworms, in China. For farmers, the economic benefits of planting Bt cotton would be eroded completely if they had to spend more money on pesticides to control secondary pests. However, Jikun Huang, a collaborator in this Chinese study, did not agree with this conclusion (J. Huang, pers. comm.). Huang explained that the rise in insecticide use for controlling secondary insects between 2001 and 2004 was due to a change in climate factors at that time. Moreover, he concluded that the increased use of insecticide needed to control secondary insects was far smaller than the total reduction in insecticide use achieved by planting Bt cotton.

### **1.4.3 Food-Human Health**

The history of TC use is too short for any long-term effects on human health to appear. There is still no strong evidence showing that TCs have direct, adverse effects on human health, even though many scientists are interested in this problem and a great deal of research has been conducted in this area. Currently, the substantial equivalence principle has been widely recognized in food safety evaluations. This means that

transgenic and traditional foods or ingredients are regarded as having the same level of human health risk, in other words that they are substantially equivalent in composition (OECD, 1993).

The health risks posed by TCs to human beings and other animals appear to be negligible. DNA from TCs, including transgenes, are digested in the stomach and processed in the intestines the same way that DNA from any other source is digested. The FDA considers food and pharmaceutical products produced from TCs to be generally recognized as safe (GRAS) (Jonas *et al.*, 2001; Goldstein *et al.*, 2005). However, plant gene fragments were demonstrated to be incorporated into the “cells that line the gastrointestinal tract and in animal immune system cells (white blood cells)” (Goldstein *et al.*, 2005). This is not likely to be a concern as there is no evidence to show that these fragments can be incorporated into an animal genome (Society of Toxicology, 2002). As Goldstein *et al.* (2005) mentioned, humans have been exposed to plant (food) DNA and gut bacterial DNA “throughout evolutionary history”.

Food allergies, including accidental exposure to allergens, are an important concern in food safety for food manufacturers and regulators (Goodman *et al.*, 2008). Sampson (2005) estimated that about 6% of young children and 3% of adults have allergic reactions to food components. GM food that mixes genes from different sources may increase the risk of allergic reactions, especially when consumers are not informed about the components in GM foods. These components may not exist naturally in the related conventional food. Furthermore, it is possible for TCs to present new allergens (Metcalf, 2003). So far, no evidence has been found that endogenous allergenicity of crops increases significantly due to genetic engineering. Also, no genes coding for allergenic proteins have been transferred into any approved commercially grown TC (Taylor, 2006). However, it is important to note that absolute

protection against all potential allergic reactions is not achievable (Goodman *et al.*, 2008) for any kind of food. In 1996, a 2S albumin gene from the Brazil nut was transferred into a transgenic soybean to increase the methionine content of animal feed made from it. This introduced protein was identified as an allergen during biosafety testing and this product was abandoned (Nordlee *et al.*, 1996).

The spread of antibiotic resistance in microorganisms is a big challenge for protecting human health. Antibiotic resistance markers (ARMs) have been used in the development of some TCs for “the preparation of plant transformation vectors or for the plant transformation process itself” (Goldstein *et al.*, 2005). However, the potential for adverse effects resulting from the use of ARMs is likely to be negligible. This is discussed further in the section following.

#### **1.4.4 Gene Flow**

“Gene flow is the natural process of movement of genes between individual organisms. Among plants this occurs mainly by pollen from one plant cross-pollinating successfully a flower from another plant and producing viable seed, a process known as outcrossing” (Glover, 2002). Gene flow occurs regularly within conventionally bred crops. The potential for gene flow between TCs and conventional crops also exists. The consequences of gene flow to ecosystems lies in the transferred genes, related traits and their interaction with the environment (Glover, 2002).

The risk of gene flow is higher between crops and their wild relatives, especially for crops grown in regions where their wild relatives are endemic, such as sunflower (*Helianthus annuus* L) and canola (*Brassica napus*) (Keeler *et al.*, 1996; Snow and Palma, 1997; Hails, 2000). Warwick *et al.* (2003) measured the frequency of gene flow from herbicide (glyphosate) resistant *Brassica napus* L. (canola) to four wild relatives in Canada and reported the first evidence of transgene outcrossing between



HT canola and one of its wild relatives, *B. rapa* L. For the other three wild relatives, the probability of gene flow was quite low ( $< 2 - 5 \times 10^{-5}$ ). The first evidence of gene flow between TCs and wild relatives in the USA was found in Oregon by Reichman *et al.* (2006). The transferred gene in glyphosate-resistant creeping bentgrass (*Agrostis stolonifera* L.) was found in nine wild bentgrass plants screened from 20,400 samples outside experimental test plots as far as 21 km beyond the perimeter of the bentgrass control area.

Quist and Chapela (2001) found the introgression of transgenic DNA constructs [the 35S promoter (p-35S) from the cauliflower mosaic virus (CMV)] in traditional maize crop varieties in Mexico, possibly from illegally planted transgenic corn. However, opponents claimed that the maize transgene results in Mexico were artifacts arising from the molecular techniques the researchers used (Kaplinsky *et al.*, 2002). In April 2002, *Nature* released an editorial note stating that “the evidence available is not sufficient to justify the publication of the original paper.” During 2003 and 2004, another study was conducted by Ortiz-Garcia *et al.* (2005) who found no detectable transgenic sequences of two transgene elements from the 35S promoter of the cauliflower mosaic virus and the nopaline synthase gene (nopaline synthase terminator) from *Agrobacterium tumefaciens* in maize seeds sampled from the state of Oaxaca in Mexico, including the same region that was sampled by Quist and Chapela (2001).

Horizontal gene transfer (HGT) from transgenic plants to bacteria has been documented to occur under ideal laboratory conditions at very low frequencies (Goldstein *et al.*, 2005). The frequency of HGT depended on the number of gene copies present in the donor and the degree of DNA homology in the recipient (Monier *et al.*, 2007). However, such transfer has not been observed out of the laboratory and remains theoretical (Goldstein *et al.*, 2005).

Additionally, there are also concerns about ARMs used in TCs to facilitate

selection of hybrids expressing the transferred genes. The spread of antibiotic resistance within and between microbial populations is a big challenge for human health. However, antibiotic resistance genes used in developing TCs are not likely to be an additional source for this problem because there is no appropriate DNA carrier molecule present in the TC that is necessary for the transfer to occur (Oeschger and Silva, 2007). Furthermore, due to “the ubiquitous nature of resistance plasmids, the far higher frequency of resistance by spontaneous mutation, and the selective effects of antibiotic use in the environment”, ARMs are unlikely to be an important means by which antibiotic resistance in bacteria is conferred, even if the transfer of ARMs from plants to bacteria is possible (Goldstein *et al.*, 2005). For free DNA in the soil, the possibility of gene flow depends on the availability of free DNA and the degree of homology between the microorganism and the free DNA. The availability of free DNA may be affected by the content and type of clay minerals and the presence of DNase. Free (naked) ARMs from tobacco (*Nicotiana tabacum* L.) and potato (*Solanum tuberosum* L.) were detected in soil for 77 and 137 d in the field, respectively (Widmer *et al.*, 1997). No transformation of free plant DNA to native soil microorganisms has been documented (Dunfield *et al.*, 2004).

#### **1.4.5 Loss of Effectiveness**

The evolution of pest resistance to TCs is a potential problem that may cause current TCs to become ineffective. With herbicide-resistant TCs, only a few particular herbicides that the TCs can tolerate are used on the weeds in crop lands. This practice may result in the development of weed species that can tolerate these particular herbicides. Farmers would then have to apply more toxic herbicides, certainly with more deleterious effects on the environment, to control the weeds that develop resistance and the current herbicide-resistant TCs would need to be abandoned.

Similarly, pest-resistant TCs will encounter inevitably the problem of the innate ability of insects to adapt to pest protection mechanisms. Griffiths *et al.* (2001) found that the nematode *C. elegans* could develop resistance to the Bt crystalline protein (Cry5B) by the loss of a galactosyltransferase, an enzyme that adds carbohydrates to proteins and lipids. This loss may make the Bt protein fail to recognize the receptor in the intestinal wall of the nematode. Some pests used in laboratory or greenhouse studies were reported to develop resistance to Bt protein (Tabashnik *et al.*, 2003). By analyzing more than a decade of monitoring data, Tabashnik *et al.* (2008) found a substantial increase in the frequency of resistance alleles in some field populations of *Helicoverpa zea* in Australia, China, Spain and the United States.

#### **1.4.6 Other Concerns**

The development of TCs, especially those resistant to environmental stresses, may retard efforts to improve environmental quality itself in some areas in which it is hard for native plants to grow, such as in saline soils (Ando and Khanna, 2000). The natural plants and ecosystems may be affected.

Lastly, there are significant religious, cultural and ethical concerns related to TCs, as well as the simple fear of the unknown (Uzogara, 2000). In these cases, additional scientific testing will not provide resolution and resistance by concerned groups to the use of TCs will continue.

### **1.5 Study Focus**

#### **1.5.1 Concerns**

The emission of greenhouse gases (GHGs) to the atmosphere, which can lead to increased global temperature, may result in serious changes in global climate. In the Kyoto Protocol, deemed the first international agreement aimed at reducing GHG

emissions, it is stated that agricultural producers can help mitigate GHG emissions by planting trees, changing crop and livestock management, and producing biofuels (McCarl and Schneider, 2000). The amount of carbon stored in soils depends on the C balance between the input from plant and animal residues and the emission from decomposition by microorganisms. In order to reduce GHG emissions to the atmosphere, more C needs to be stored in soils. This can be realized by increasing the C input and/or decreasing decomposition of existing C stores (Paustian *et al.*, 2001).

As mentioned above, conflicting results for both lignin concentration and decomposition rate of Cry1Ab corn residues have been reported. Changes in the lignin concentration and the rate of plant residue decomposition in the field may have effects on GHG emissions and C sequestration in soils. The degradation of Bt protein in residues is another concern as this may determine the exposure level of non-target organisms to Bt protein in environment. Different C allocations to roots and shoots can lead to different C inputs to soils. Because of “the continuous release of C from roots and the complex nature of the rhizosphere-soil interface” (Puget and Drinkwater, 2001), plant roots may contribute more to stored soil C than the aboveground biomass (Boone, 1994; Norby and Cotrufo, 1998). The factors that could affect C allocation within plants include genotypic considerations (Vessey, 1992; Percival *et al.*, 2001) and the presence of pathogens or pests (Feldman, 1984; Wild, 1988; Waisel *et al.*, 1991). The Cry3Bb Bt corn hybrids are protected from root damage by corn rootworm (CRW) but the NonBt hybrids do not have such traits. This may lead to plant phenotypes that allocate C differently.

The Office of Genetic Engineering Safety Administration (OGESA) in the Ministry of Agriculture (MOA) in China is considering approving the commercial release of IR rice. As rice is a staple food in China, this possible approval will have a more profound effect on people’s lives than any other TC. Consumers’ perceptions

will be among the most important factors determining the acceptance and economic success of commercially released IR rice in the market. Farmers' attitudes and their willingness to adopt Bt rice are also critical for its success because farmers are more involved in this issue than any other group. Attitudes of farmers and consumers need to be considered in the governmental decision-making process.

### **1.5.2 Study Aims and Hypotheses Tested**

In my study, I worked in both the greenhouse and the field to compare Cry3Bb Bt corn, resistant to the corn rootworm, with its non-transgenic isoline. I aimed to determine the relative field decomposition rates and lignin concentration of Bt and NonBt corn residues; the field decomposition of the Cry3Bb protein; the relative contributions of the presence of the Bt transgene and pest pressure on C allocation and lignin concentration or content in different corn plant parts in greenhouse trials; and the possible effects of Bt and NonBt residues on microbial decomposer populations colonizing residues in the field.

- (1) H<sub>0</sub>: Bt corn residues decompose in the field at the same rate as those of the corresponding NonBt corn isoline.
- (2) H<sub>0</sub>: The composition of the microbial community colonizing the residues of Bt and NonBt corn do not differ.
- (3) H<sub>0</sub>: Pest feeding on NonBt corn roots will have no effect on C allocated to roots as compared with Bt corn protected from pest feeding by the expression of the Cry3Bb protein in root tissue.
- (4) H<sub>0</sub>: Differences in plant biomass between corn varieties in different maturity groups is not greater than the difference between Bt and NonBt corn within the same maturity group.

(5) H0: Lignin concentration or content of Bt and NonBt corn residues does not differ, regardless of whether plants are grown in the greenhouse or the field.

Beyond these scientific studies, I evaluated the effects of scientific findings on people's perceptions towards transgenic food, especially transgenic rice, in China by use of surveys and interviews and used this information to predict future scenarios. The main purposes of the survey were to investigate the awareness and attitude structures of Chinese consumers and farmers in order to provide useful information to government policy-makers and the TC industry regarding the potential commercial release of IR rice. The factors that may affect respondents' attitudes were also explored.

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**CHAPTER 2**  
**DECOMPOSITION RATE AND MICROBIAL COMMUNITIES**  
**COLONIZING RESIDUES DO NOT DIFFER BETWEEN Cry3Bb BT AND**  
**NONBT CORN HYBRIDS IN THE FIELD**

**Abstract**

Transgenic corn variety MON863 produces the Cry3Bb protein from *Bacillus thuringiensis* (Bt), which has insecticidal activity against the corn rootworm complex. MON863 was released for commercial use in the U.S. in 2003. Despite the rapid adoption of Bt crops by farmers, public concern continues to mount over the potential environmental impacts of these crops. Reduced rates of residue decomposition and increased lignin concentration have been highlighted most recently. In this study, I assessed the effects of Bt and NonBt corn on rates of residue decomposition, tissue lignin concentration and associated decomposer communities. Litterbags containing cobs, roots, or stalks plus leaves from Bt (MON863) and NonBt corn without and with insecticide applied (NonBt+I), were placed on the soil surface and at 10 cm depth in field plots that corresponded to these crop treatments. Initial acid detergent lignin content was measured for the residues used to prepare the litterbags. After 3.5, 15.5 and 25 months, litterbags were collected from the field and DNA was extracted from the microbial communities that colonized the residues in the litterbags. Fungal and bacterial community DNA was amplified by PCR with the internal transcribed spacer (ITS) and 16S rRNA gene primers, respectively. PCR products were digested in separate reactions using the restriction enzymes HhaI and MspI for fungi, and HhaI and Sau96I for bacteria. Terminal restriction fragments were sized and the resulting data analyzed by use of multivariate statistical approaches. There was no significant effect of treatment (Bt, NonBt and NonBt+I) on initial tissue lignin concentrations,

litter decomposition rate or bacterial communities colonizing the residues in litterbags. The effect of treatment on fungal communities colonizing the residues in litterbags was small, with only one of 16 comparisons yielding separation by treatment (2006 buried residue samples from AR plots for the HhaI digests). Lignin concentration and litter decomposition rate differed significantly by plant part, but microbial communities colonizing these residues did not. Environmental factors, including season, litterbag placement (surface vs. buried) and plot history, led to significant differences for most variables measured in this study. Combined, these results indicate that differences in measured variables were driven primarily by environmental factors rather than by any differences between the corn hybrids (genotypes) or the use of the insecticide tefluthrin. The Cry3Bb corn tested in this study did not affect tissue lignin concentrations, residue decomposition rates, or microbial communities colonizing the residues, thus is unlikely to affect C turnover or sequestration in soil.

## **2.1 Introduction**

Transgenic corn variety MON863 that produces the Cry3Bb protein from *Bacillus thuringiensis* (Bt), which has insecticidal activity against the corn rootworm (CRW) complex (*Diabrotica* spp.) (Coleoptera), was released for commercial use in the U.S. in 2003 (USEPA, 2003). The adoption of Cry3Bb corn in agriculture may reduce the conventional practice of spraying pesticides on crops, which has serious detrimental effects on human health and the environment (Ando and Khanna, 2000), and achieve some environmental, economic, health and social benefits (James, 2004). However, concerns continue to be raised by the public about the potential environmental and health effects of transgenic crops (TCs). As other breeding methods do, genetic engineering may also lead to pleiotropic effects on macroscopic plant characteristics, which could have ecological implications (Tian and Chen, 2001). Altered lignin

concentrations and associated slower rates of residue decomposition have been highlighted most recently (Saxena and Stotzky, 2001; Castaldini *et al.*, 2005; Flores *et al.*, 2005; Fang *et al.*, 2007; Zwahlen *et al.*, 2007; Lehman *et al.*, 2008). Altered rates of plant residue decomposition in the field is a major concern in view of its potential effects on nutrient cycling, greenhouse gas (GHG) emissions and carbon (C) sequestration in soils.

Conflicting results for both lignin concentration and decomposition rate of Cry1Ab corn residues have been reported. The information for Cry3Bb corn is still limited. Saxena and Stotzky (2001) found that stalks of Cry1Ab Bt corn (Bt11, MON810 and Bt176) grown in a plant growth room or in the field, had a significantly higher (33–97%) lignin concentration than that of their parental varieties without the *cry* protein gene (NonBt isolines), which they suggested could account for the slower decomposition rates they observed for the Bt corn residues. Other studies confirmed that Bt Cry1Ab corn had a slower decomposition rate (Castaldini *et al.*, 2005) or higher lignin concentration (Flores *et al.*, 2005) or both (Flores *et al.*, 2005). However, results from other studies show that the ability to produce the Cry1Ab protein had no significant effect on lignin concentration or residue decomposition rate (Hopkins and Gregorich, 2003; Jung and Sheaffer, 2004; Zwahlen *et al.*, 2007; Tarkalson *et al.*, 2008). Lehman *et al.* (2008) found that there was no difference in field decomposition rates or lignin concentration in Cry1Ab, Cry3Bb, and stacked Cry1Ab/Cry3Bb Bt corn hybrids compared to their non-transgenic counterparts. A lower lignin concentration was found in Cry1Ab Bt corn (X4334-EPR, Novartis, previously Northrup King) leaves during the decomposition process between 2 and 4 weeks after putting soaked leaves in plastic boxes without soil (Escher *et al.*, 2000). As a soil-free system was used, however, this does not necessarily reflect the natural process of decomposition in field soil. It is important to note that most studies mentioned above used the term

“lignin content”, but they actually measured and reported the lignin concentration (as % or g kg<sup>-1</sup>).

Microorganisms are responsible for performing many critical soil ecosystem functions, including nutrient cycling and residue decomposition. Bt protein released from roots and residues of Bt corn in the field could affect the activity of decomposer microbial communities. Saxena *et al.* (1999) found that Cry1Ab protein released in root exudates could bind to surface-active particles in soil, which is likely responsible for its slower biodegradation and persistence in soil. Tapp and Stotzky (1998) found that the insecticidal activity of the protein remained after adding purified Cry1Ab protein to non-sterile soils and incubating for 234 days. However, the Cry3Bb1 protein was not detected in soil planted to Bt corn for one or three consecutive years (Ahmad *et al.*, 2005). Icoz and Stotzky (2007) conducted a laboratory study and found that Cry3Bb1 protein degraded rapidly and didn't persist or accumulate in soil. However, they suggested further studies since soils differ in their physicochemical and biological characteristics and these are likely to be important factors determining the persistence of the protein in different soils and locations. Residues decomposer communities could also be influenced by the physical and chemical nature of plant materials (Collins *et al.*, 1990). Possible differences in lignin concentration in Cry3Bb Bt corn residues may lead to changes in microbial communities colonizing the residues.

In this study, I aimed to evaluate the effects of Cry3Bb Bt corn residue, its NonBt isoline, and the NonBt isoline treated with the insecticide tefluthrin on rates of residue decomposition, initial plant lignin concentration and the composition of microbial communities colonizing the residues in the field.

## **2.2 Materials and Methods**

### **2.2.1 Field Plots**

Field trials were established at the Cornell University's Musgrave Farm in Aurora, NY, and continued for 3 consecutive seasons from 2004 - 2006. The main treatments were: (1) MON863 corn producing the Cry3Bb protein (Bt); (2) a non-transgenic isoline (NonBt), and (3) the NonBt isoline with tefluthrin insecticide Force 3G applied at planting (NonBt+I). The field soil is a Lima loam: 43.8% sand, 37.4% silt, 19% clay, with a pH of 7.4, and 4.6% organic matter.

Seeds of MON863 and its NonBt isoline were obtained from Monsanto Corp. (St. Louis, MO). The tefluthrin insecticide Force 3G (AstraZeneca Corp, Wayne, PA), a pyrethroid insecticide used commonly to control insect pests of corn, was applied to half of the NonBt corn plots to simulate a typical agricultural practice in the absence of the Bt crop.

The treatments were established in plots continuously planted to corn (CC) and duplicated in another location within the same farm previously rotated out of alfalfa (AR). Prior to the initial planting of this experiment, the CC plots were found to have CRW present in sufficient numbers to damage corn roots (Leslie Allee, Entomology, Cornell University, pers. comm.), while the AR plots had little or no CRW present in the first year.

Each treatment had three replicate plots (50 x 50 m). A randomized complete block (RCB) design was applied. Blocks established in the AR plots were contiguous; whereas the remaining blocks for the CC plots were not. For blocks established in fields where continuous corn was grown, one was adjacent to the AR blocks and the other two were within 200 m.

The core experiment ended in May 2007. After this time, all plots were planted



with oats, except the CC block which was adjacent to the AR blocks. However, litterbags remained in the field until August, 2007.

### **2.2.2 Residue Decomposition**

Corn cobs, shoots (stalks + leaves) and roots were collected separately from the field after harvest in 2004 and stored at 4°C. Residues were oven-dried (65°C) to a constant weight and then used in litterbag studies. Separate mesh bags (12.5 x 12.5 cm; 2 x 3 mm mesh size ellipse) were filled with 7 g of cobs, 10 g of shoots or 5 g of roots with two duplicate bags for each retrieval time point. In litterbags containing shoots, the ratio of stalks to leaves was 3:2 and was determined by measuring the ratio of these two components in the field subsamples collected. Litter bags were placed on the soil surface or at 10 cm depth in the field on June 24, 2005. Each spring when the plots were tilled prior to planting and each fall when the plots were harvested, the litterbags were removed from the field and stored at 4°C for about 2 weeks until those activities were completed and then replaced. Litterbags were sampled on October 10, 2005, October 19, 2006, and August 3, 2007, after 3.5, 15.5 and 25 months in the field, respectively.

In 2005, after the litterbags were recovered from the field, DNA was extracted from residue surface biofilms for one set of litterbags. Arthropods were extracted from the other set of litterbags using the Tullgren funnel method at the New York State Agricultural Experiment Station in Geneva, NY (data not shown). After DNA or arthropods were extracted, litterbags were dried to a constant weight in a 65°C oven and then ground in an ED-5 Thomas Wiley mill grinder (Arthur H. Thomas, Co., PA) with a sieve size of 2 mm. Approximately 0.5 g of each ground subsample was placed in a borosilicate tube and put into a 450°C muffle furnace for 7 h to determine the ash-free-dry-weight (AFDW).

In 2006, after duplicate litterbags were recovered from the field, AFDW was measured directly for one set of litterbags and DNA was extracted from the surface biofilms for the other set of litterbags. Buried root samples were separated from soil using a particulate organic matter (POM) extraction (Christensen, 1992). A series of 12.7 cm (5-inch) diam nesting sieves were stacked in order of 2 mm, 250  $\mu$ m, 53  $\mu$ m opening from top to bottom. Litterbag residues were placed on the top sieves and then rinsed through the sieves with water. Remaining materials in all three sieves were transferred into a container and POM was collected by density fractionation. POM was placed into a 65°C oven for one week and AFDW was obtained as described above.

In 2007, AFDW was also measured directly for one set of litterbags. The POM recovery method was used to recover remaining residues in buried litterbags for all plant parts.

### **2.2.3 Initial Lignin Concentration**

Dry cobs, shoots and roots, collected at harvest in 2004 and used to prepare the litterbags, were ground and then analyzed for acid detergent lignin (ADL) concentration following the protocol of Goering and Van Soest (1975). Approximately 1 g of each ground sample was weighed and placed in a 600 mL Pyrex beaker. One hundred milliliters of acid detergent solution (composed of 1N sulfuric acid, technical grade cetyl trimethylammonium bromide and 2% decahydronaphthalene) were added to each beaker, which was then connected to a refluxing apparatus and heated for 1 h. The materials were filtered by vacuum and transferred into a Gooch crucible. The residues were then washed with acetone to remove all color and break up lumps and put into an oven (100°C) overnight to dry. The acid detergent fiber (ADF) was calculated as follows:

$$\text{ADF} = (W_0 - W_t) (100) / S$$

Where:  $W_0$  = weight of oven-dry crucible including fiber

$W_t$  = tare weight of oven-dry crucible

$S$  = oven-dry sample weight

Crucibles containing the ADF were half-filled with cooled (15°C) 72%  $H_2SO_4$  and stirred with a glass rod to break all lumps. The crucible was re-filled with 72%  $H_2SO_4$  three times at hourly intervals and then kept at 20° to 23°C for 3 h. After that, materials were filtered by vacuum and washed with hot distilled water 5 times to remove the acid. Final filtration was done by adding acetone to the crucible and drying overnight in a 100°C oven. Hot crucibles containing the residues were weighed, ignited for 3 h in a 520°C muffle furnace, cooled to 100°C and then weighed again. The acid detergent lignin (ADL) was calculated as follows:

$$ADL = (L \times 100) / S$$

Where:  $L$  = loss upon ignition after 72%  $H_2SO_4$  treatment

$S$  = oven-dry sample weight

#### **2.2.4 Microbial Communities Colonizing Residues in Litterbags**

The terminal restriction fragment length polymorphism (T-RFLP) method was used to characterize residue decomposer communities.

Biofilms were detached from the residue surfaces by placing residues from litterbags in vials containing 20 mL 0.5 M sterile potassium phosphate buffer (pH 6.8). The vials were shaken strongly in a mechanical shaker for 1 hr. The biofilm suspension was then dispensed into 2 mL tubes and centrifuged until a dense pellet of biomass was obtained. DNA was extracted from approximately 300  $\mu$ L of this biomass using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc, Carlsbad, CA). The DNA was then quantified by measuring fluorescence of ethidium bromide bound to DNA and comparing it against a standard curve prepared with calf thymus

DNA (Trevigen Inc, Gaithersburg, MD) using Quantity One Software (BioRad, Hercules, CA).

Polymerase chain reaction (PCR) amplification of the extracted DNA was performed using both bacterial and fungal universal primers. For the bacterial analysis, fluorescently labeled 27 forward primer 5'-/6-FAM/AGA GTT TGA TCC TGG CTC AG-3' and 1492 reverse primer 5'-GGT TAC CTT GTT ACG ACT T-3'; synthesized by Integrated DNA Technologies (Coralville, IA) was used to amplify 16S rRNA genes, resulting in products of approximately 1500 bp (Lane, 1991). Each 50  $\mu$ L PCR reaction contained 7.5 ng of DNA template, 1x PCR buffer, 2.0 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs, 0.1 mg  $\text{mL}^{-1}$  bovine serum albumin (BSA), 0.1  $\mu$ M of each primer, and 0.05 U  $\mu\text{L}^{-1}$  of Taq polymerase (all from Promega, Madison, WI). DNA was amplified in an MJ Research thermal cycler using an initial denaturing step of 94°C for 3 min followed by 30 cycles of the following program: denaturation at 94°C for 30 s, primer annealing at 59°C for 45 s, and extension at 72 °C for 60 s. A final extension at 72°C for 15 min was performed after the thermal cycling was completed. For the fungal analysis, the internal transcribed spacer (ITS) region between the 18S and 28S rRNA gene regions was amplified using 0.2  $\mu$ M of both fluorescently labeled ITS1 forward 5'-/6-FAM/TCC GTA GGT GAA CCT GCG G-3' and ITS4 reverse primers 5'- TCC GTA GGT GAA CCT GCG G-3', resulting in products of approximately 600 bp (White *et al.*, 1990). Each 50  $\mu$ L PCR reaction contained 15 ng of DNA template, 0.05 U  $\mu\text{L}^{-1}$  of Taq polymerase, 3.0 mM  $\text{MgCl}_2$ , 1x PCR buffer, 0.6 mM dNTPs, 0.1 g  $\text{L}^{-1}$  BSA, and nuclease free water. As described for bacteria, 50  $\mu$ L reactions were performed. The PCR program consisted of an initial denaturing step at 94°C for 5 min, followed by 30 cycles of the following program: denaturation at 94°C for 30 s, annealing at 51°C for 45 s, and extension at 72 °C for 45 s. A final extension at 72°C for 10 min was performed. The PCR reactions for each sample were separated in a

1.5% agarose gel to verify the products, which were then quantified as described above. A 300 ng subsample of each PCR product was digested in separate reactions using the restriction endonucleases HhaI and MspI (for fungi) and HhaI and Sau96I (for bacteria) in 30  $\mu$ L reactions containing: 300 ng of DNA, 1 U of restriction enzyme, 3  $\mu$ L of the appropriate 10 x buffer, and 0.002 mg  $\mu$ L<sup>-1</sup> BSA. The reactions were incubated at 37°C for 4.5 h, followed by an inactivation step at 68°C for 15 min. The restricted products were purified using Performa DTR Edge plates (Edge BioSystems, Gaithersburg, MD), then evaporated and re-suspended in 10  $\mu$ L of a mixture containing 9.9  $\mu$ L of 99% formamide and 0.1  $\mu$ L of 500 LIZ size standard. The length of the fluorescently-labeled terminal fragments was determined using an ABI3730 X1 capillary DNA sequencer (Life Sciences Core Facility, Cornell University, Ithaca, NY).

Electropherograms obtained from the ABI3730 X1 were analyzed using GeneMapper 3.0 software (Applied Biosystems, 2004). Only those bands sized between 50 and 500 bp were used for statistical analysis using MATMODEL software (Microcomputer Power, Ithaca, NY) to generate Additive Main effects and Multiplicative Interactions (AMMI) analyses.

### **2.2.5 Statistical Analysis**

Analysis of variance (ANOVA) with a linear mixed-effect model was conducted using S-PLUS 8.0 (TIBCO Software Inc., Palo Alto, CA). Block was set as the random factor. All other treatment variables were set as fixed factors.

## **2.3 Results**

### **2.3.1 Initial Lignin Concentration**

The initial lignin concentration of the 2004 corn residues used to prepare the litterbags is shown in Figure 2.1. There was no significant effect of treatment (Bt, NonBt or NonBt+I) on lignin concentration in the residues, but plant part was a significant factor ( $p < 0.0001$ ) (Table 2.1). The order of plant parts from highest to lowest lignin concentration was roots > stalks > cobs > leaves. Roots had the highest lignin concentration (9.9 - 13.3%). Leaf lignin concentration was the lowest of all plant parts and ranged from 4.2 - 6.7%. Based on the ratio of 3:2 (stalks:leaves), the lignin concentration of mixed litterbags ranged from 4.7 - 12.5%.

There was a significant interaction between plant part and plot history for lignin concentration in the ANOVA analysis ( $p = 0.04$ ) (Table 2.1). This could be attributed to the significantly higher lignin concentration in CC cobs than in those cobs harvested from AR plots.

### **2.3.2 Residue Decomposition**

Residue mass loss over time was determined by measuring the weight of litter remaining (%) in the litterbags at each sampling time based on ash free dry weight (AFDW) (Figure 2.2). After 25 months in the field, an average of 90.8% of buried cobs and 95.5% of buried shoots were decomposed, while 68.3% of surface-placed cobs and 79.4% of surface-placed shoots were decomposed. For all three sampling times, treatment (Bt, NonBt or NonBt+I) was not a significant factor affecting decomposition in the ANOVA analysis (Table 2.2). The weight remaining of Bt, NonBt and NonBt+I litterbags was 63.67 ( $\pm 2.98$ )%, 62.29 ( $\pm 3.02$ )% and 64.89 ( $\pm 2.99$ )%, respectively, after 3.5 months; 27.94 ( $\pm 3.99$ )%, 23.98 ( $\pm 2.61$ )% and 26.63

( $\pm 3.86$ )%, respectively, after 15.5 months; 19.21 ( $\pm 3.43$ )%, 16.34 ( $\pm 3.15$ )% and 15.40 ( $\pm 2.36$ )% ( $\pm$ SE), respectively, after 25 months.

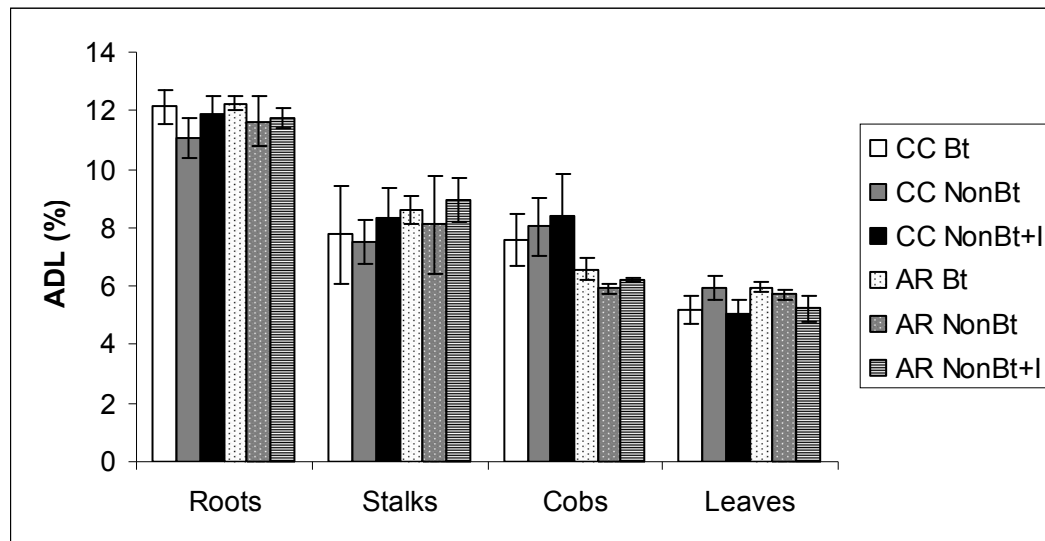


Figure 2.1 Initial Acid Detergent Lignin (ADL) concentration in the corn tissues used to prepare litterbags (error bars: SE).

Table 2.1 ANOVA for initial acid detergent lignin (ADL) concentration.

Source of Variance	ADL Concentration		
	df	F-value	p-value
Treatment (T)	2	0.27	0.77
Plot history (H)	1	0.29	0.59
Plant part (P)	3	68.55	<b>&lt;0.0001</b>
T $\times$ H	2	0.31	0.74
T $\times$ P	6	0.43	0.86
H $\times$ P	3	2.92	<b>0.04</b>
T $\times$ H $\times$ P	6	0.12	0.99

For all sampling times, depth of litterbag placement was a significant factor ( $p < 0.0001$ ) for weight of residue remaining in the ANOVA analysis. Buried samples decomposed faster than those placed on the soil surface. On average, the weight remaining of surface-placed cobs and shoots was 37% and 46% higher than their corresponding buried samples, respectively, after 3.5 months. After 15.5 months, the

weight remaining of surface-placed cobs and shoots was 1.9 and 3.7 times more than the corresponding buried samples, respectively. These ratios increased to 3.4 and 4.5 times after 25 months in the field for cobs and shoots, respectively.

Plant part affected residue decomposition rate significantly for all three sample times ( $p = 0.04$ ,  $0.0002$  and  $0.0007$  in 2005, 2006 and 2007, respectively). Weight remaining of cobs was 10.1, 55.1 and 59.2% higher than that of shoots after 3.5, 15.5 and 25 months, respectively. The weight of surface-placed roots collected in 2005 was heavier than the initial weight, indicating possible soil and/or new roots infiltrating the litterbags. The weight remaining of surface-placed root litterbags in 2006 decreased, but was still higher than the initial weight. In 2007, the weight of surface-placed roots was  $85.3 (\pm 8.6)\%$  ( $\pm$  SE) of the initial weight. The buried root data are shown in Figure 2.2, although weight remaining of Bt and NonBt+I samples was still higher than their initial weights after 3.5 months. Generally, the order from fastest to slowest mass loss was: shoots > cobs > roots.

Apart from the root data, the interaction between plant part and plot history on weight remaining was significant at  $p = 0.08$  in 2005 and at  $p < 0.05$  in 2006 and 2007 (Table 2.2). In 2006, weight remaining of surface-placed shoot samples in CC plots was 35.8% lower than in AR plots. In 2007, weight remaining of all buried samples (cobs, shoots and roots) from CC plots was 52.9% lower than from AR plots. For 2007 surface-placed samples, only the weight remaining of shoots from CC plots was 38.9% lower than that from AR plots.



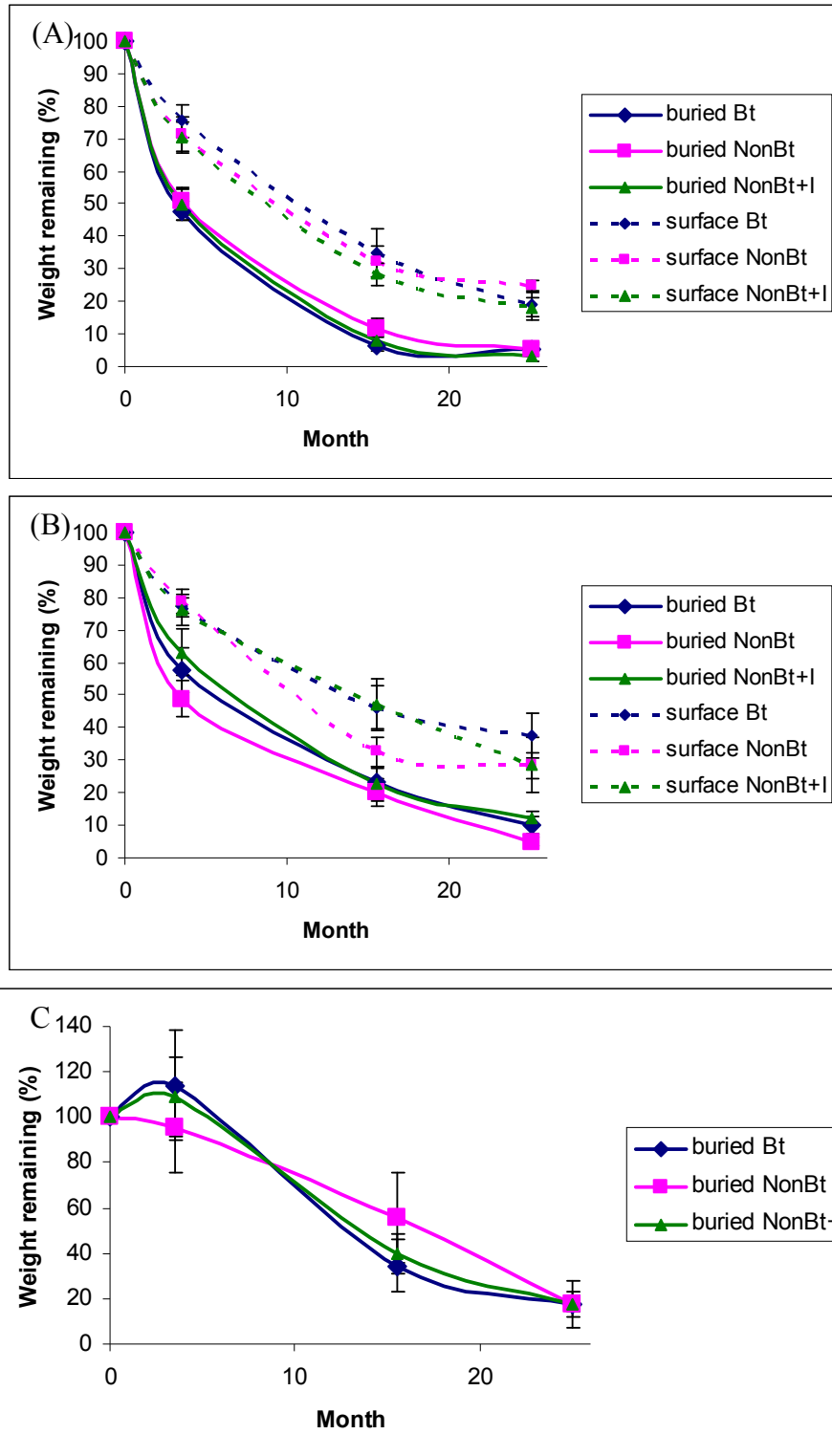


Figure 2.2 Decomposition of litterbags containing (A) shoots; (B) cobs; (C) roots (error bars: SE).

Table 2.2 ANOVA for weight remaining in 2005, 2006 and 2007.

Source of Variance	df	2005		2006		2007	
		F-value	p-value	F-value	p-value	F-value	p-value
Plant part (P)	1	<b>4.39</b>	<b>0.04</b>	<b>16.64</b>	<b>0.0002</b>	<b>13.26</b>	<b>0.0007</b>
Depth (D)	1	<b>56.79</b>	<b>&lt;.0001</b>	<b>61.28</b>	<b>&lt;.0001</b>	<b>82.65</b>	<b>&lt;.0001</b>
Plot history (H)	1	0.10	0.75	0.65	0.42	1.38	0.25
Treatment (T)	2	0.23	0.80	0.60	0.56	0.50	0.61
P × D	1	0.11	0.75	0.37	0.55	2.34	0.13
P × H	1	<b>2.98</b>	<b>0.09</b>	<b>5.08</b>	<b>0.03</b>	<b>5.47</b>	<b>0.02</b>
P × T	2	0.48	0.62	1.80	0.18	2.05	0.14
D × H	1	1.06	0.31	2.84	0.10	1.32	0.26
D × T	2	0.79	0.46	1.14	0.33	0.89	0.42
H × T	2	0.91	0.40	1.69	0.20	0.19	0.82
P × D × H	1	0.58	0.45	0.14	0.71	<b>5.58</b>	<b>0.02</b>
P × D × T	2	0.92	0.40	0.40	0.67	0.66	0.52
P × H × T	2	0.31	0.73	0.04	0.96	1.04	0.36
D × H × T	2	1.12	0.33	1.85	0.17	1.44	0.25
P × D × H × T	2	0.11	0.89	0.24	0.79	2.67	0.08

### 2.3.3 Microbial Communities Colonizing Residues in Litterbags

Bacterial and fungal community composition was determined by T-RFLP analysis for the independent variables of treatment (Bt, NonBt or NonBt+I), years (2005 or 2006 season), plot history (CC or AR), plant parts (cob, root, shoot), and litterbag placement (buried or surface-placed). In the AMMI analysis, the percents of predicted interaction signal variation captured in the first interaction principal component (IPCA 1), which represented year of sampling, were all above 41.9% (Figure 2.3, 2.4). However, for 2005 bacteria and 2006 fungal community terminal restriction fragments (TRFs) resulting from use of the HhaI enzyme (Figure 2.5 A, 2.6B), this percentage exceeded 100%, indicating that IPCA 1 recovered selectively all predicted signal and contained predicted interaction noise. Results from use of Sau96I and MspI restriction enzymes (data not shown) show similar trends to those obtained from use of the HhaI restriction enzyme.

### **2.3.3.1 Bacterial Communities**

There was no clear separation of samples based on treatment (Bt, NonBt and NonBt+I) (Figure 2.3A), or plant part (cob, shoot and root) for either enzyme (HhaI or Sau961), indicating that neither residue treatment nor plant part were the primary variables controlling residue colonizing bacterial community composition.

However, samples separated clearly by year (2005 or 2006) for both enzymes, indicating that bacterial communities differed between years. For the HhaI enzyme (Figure 2.3 B), the samples also separated by depth (buried or surface-placed). The same trend was observed for the Sau961 digestions, but not as clearly as for digestions with the HhaI enzyme. In the AMMI analysis, variation along IPCA1 was associated with year, whereas litterbag placement explained most of the variation along IPCA2. This indicates that year was the first main factor affecting bacterial communities and depth of residue placement was the second.

For TRFs resulting from HhaI digestions (Figure 2.3 B), buried samples from 2005 separated clearly by plot history. The 2006 samples and 2005 surface-placed samples tended to separate by plot history (CC vs AR), but not as clearly. However, when analyzing the data individually by year (Figure 2.5 A), the effect of plot history was evident for both the buried and surface-placed residue samples for the HhaI digests, but less so for Sau961 digests.

### **2.3.3.2 Fungal Communities**

Fungal ITS TRFs derived from HhaI digests separated into three groups along IPCA1: (1) all 2005 samples, 2006 surface-placed samples and buried NonBt, NonBt+I samples from AR plots; (2) 2006 buried Bt samples from AR plots; (3) 2006 samples from CC plots (Figure 2.4). For MspI digests, samples clearly separated into two groups by year (2005 and 2006).

Terminal restriction fragments separated by treatment (Bt, NonBt or NonBt+I) for only one of 16 comparisons across the entire experiment (2 seasons, 2 depths, 2 plot histories and 2 enzymes) for the 2006 buried samples (cobs, shoots and roots) from AR plots for Hha1 digests. No clustering or separation by treatment was found for the rest of samples. There was also no clear separation by plant part (cobs, shoots or roots) for either enzyme (Hha1 or Msp1), indicating that fungal communities colonizing different residue types did not differ strongly.

Samples separated clearly by year (2005 and 2006) for both Hha1 and Msp1 digests, indicating that the fungal communities differed between years. For Hha1 digests, 2006 samples from AR plots still separated clearly from 2005 samples, although there was some overlap (Figure 2.4 B).

Samples separated clearly by depth of litterbag placement for both the Hha1 (Figure 2.4 B) and Msp1 digests (data not shown). The influence of plot history was also clear with a single exception, a 2005 surface-placed sample for Hha1 digests. Despite this exception, when analyzing the 2005 data for Hha1 digests alone (Figure 2.6 A), surface-placed samples separated clearly by plot history.

The analysis of fungal communities within years (2005 or 2006) is shown in Figure 2.6. The 2005 residue-colonizing fungal communities separated clearly by depth along IPCA1, and then by plot history along IPCA2 (Figure 2.6 A for Hha1). However, for the 2006 samples, plot history was the major variable explained along the IPCA1 axis (Figure 2.6 B). This indicated that the first dominant factor affecting fungal communities was depth of litterbag placement in 2005 and plot history in 2006.

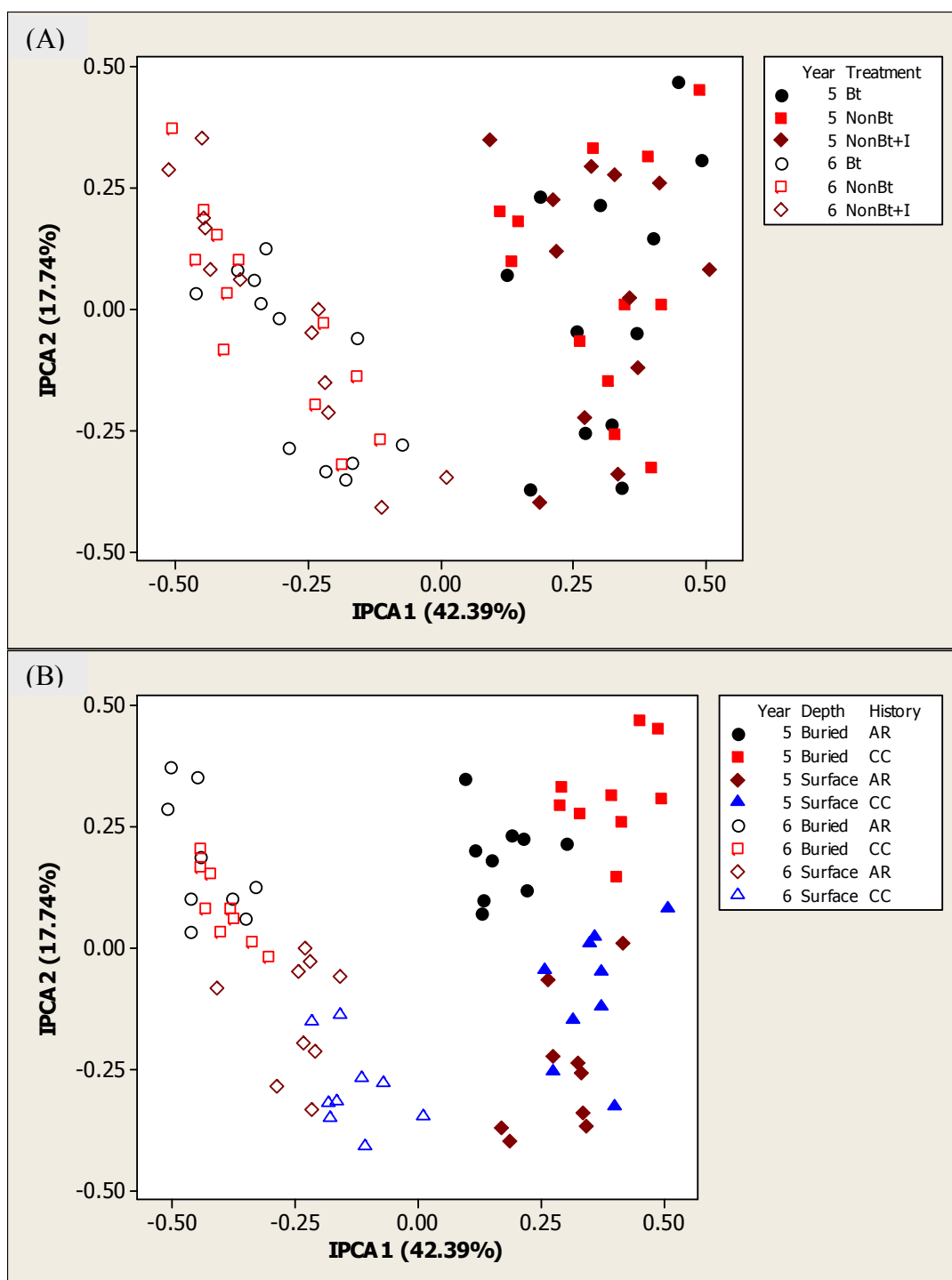


Figure 2.3 Bacterial communities colonizing residues in litterbags, HhaI restriction enzyme, labeled by (A) year (5=2005, 6=2006) x treatment; (B) year x depth x plot history.

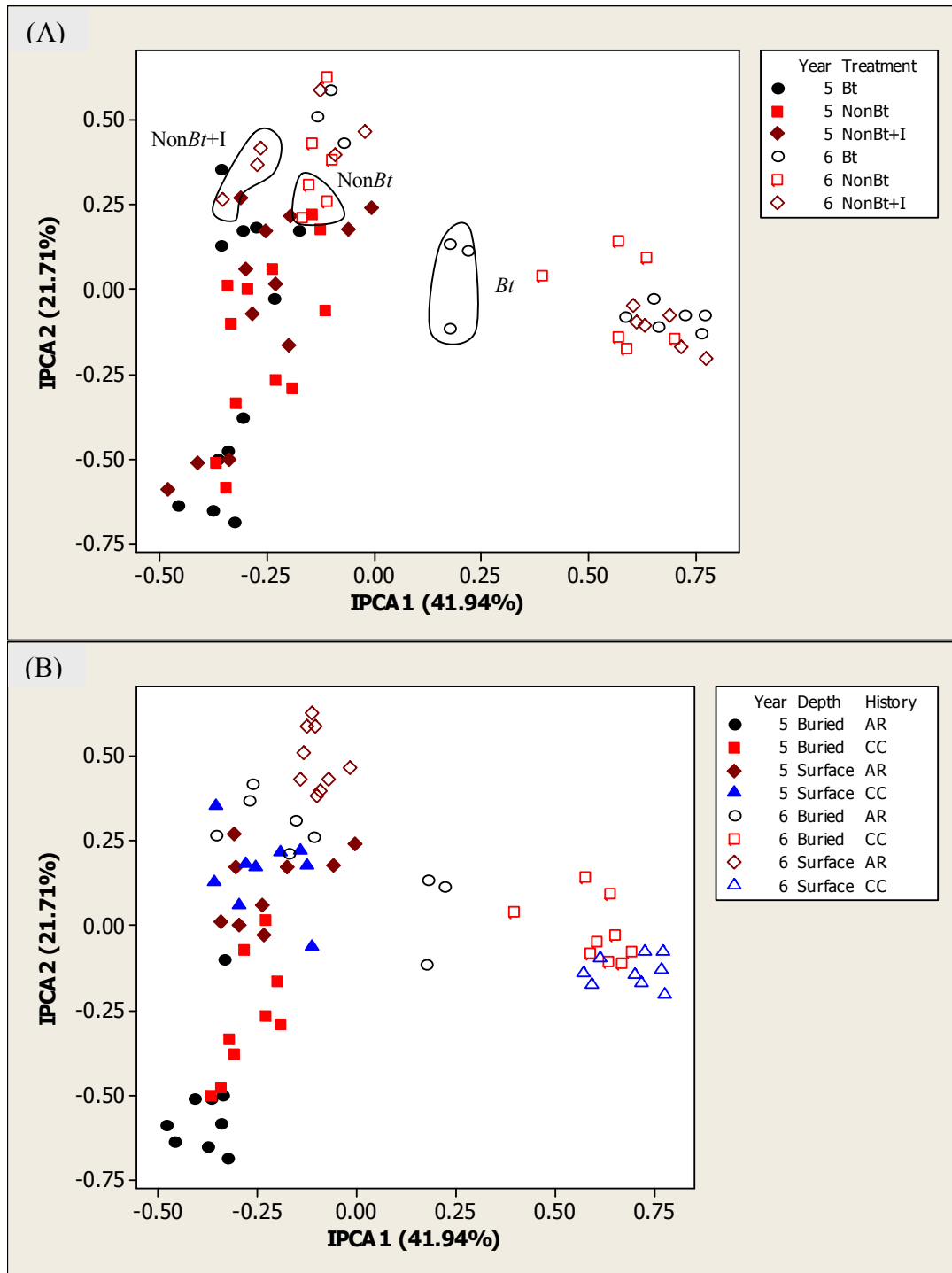


Figure 2.4 Fungal communities colonizing residues in litterbags, HhaI restriction enzyme, labeled by (A) year (5=2005, 6=2006) x treatments; (B) year x depth x plot history.

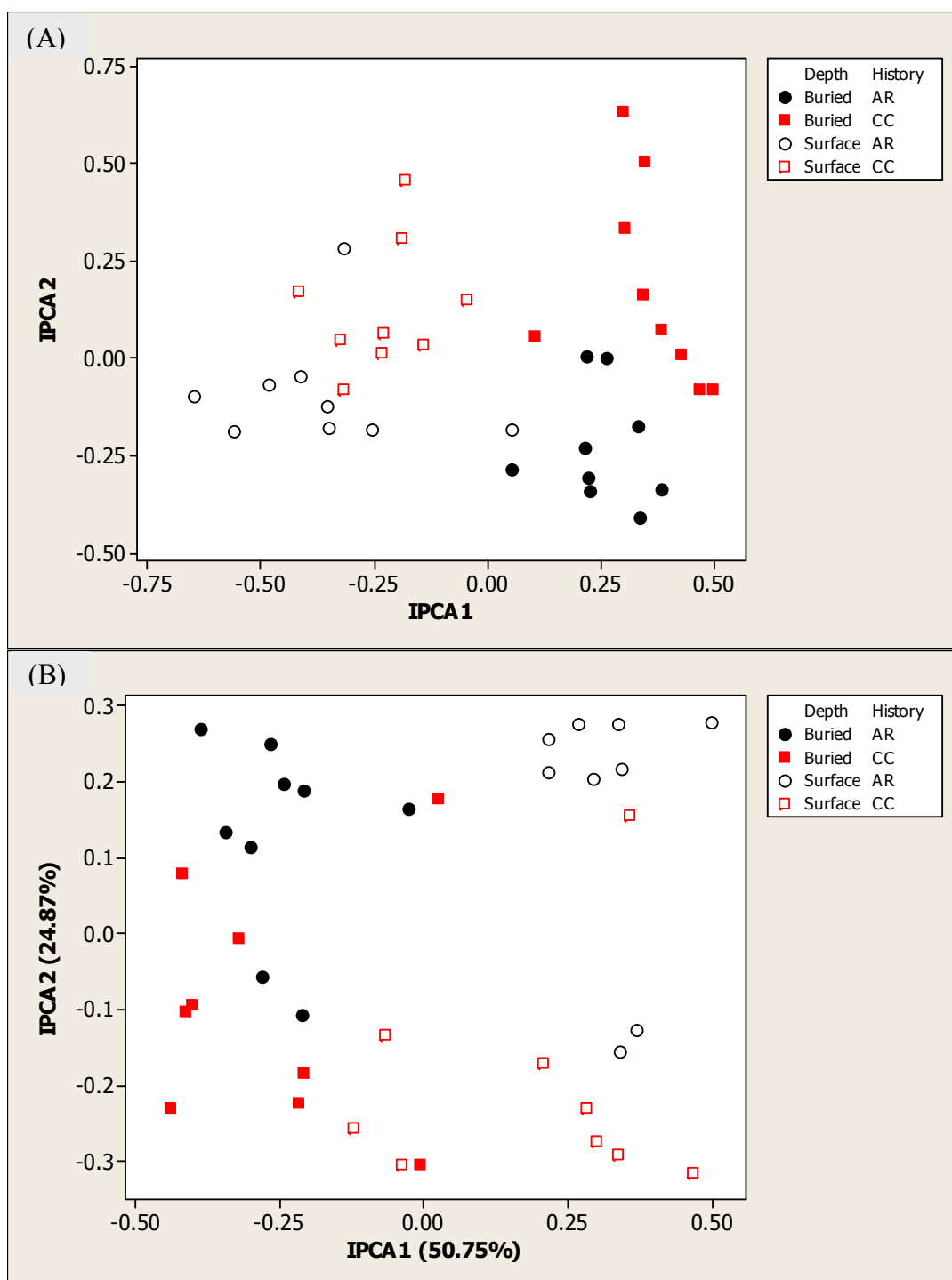


Figure 2.5 Bacterial communities colonizing residues in litterbags, Hha1 restriction enzyme, labeled by depth and plot history in (A) 2005; and (B) 2006.

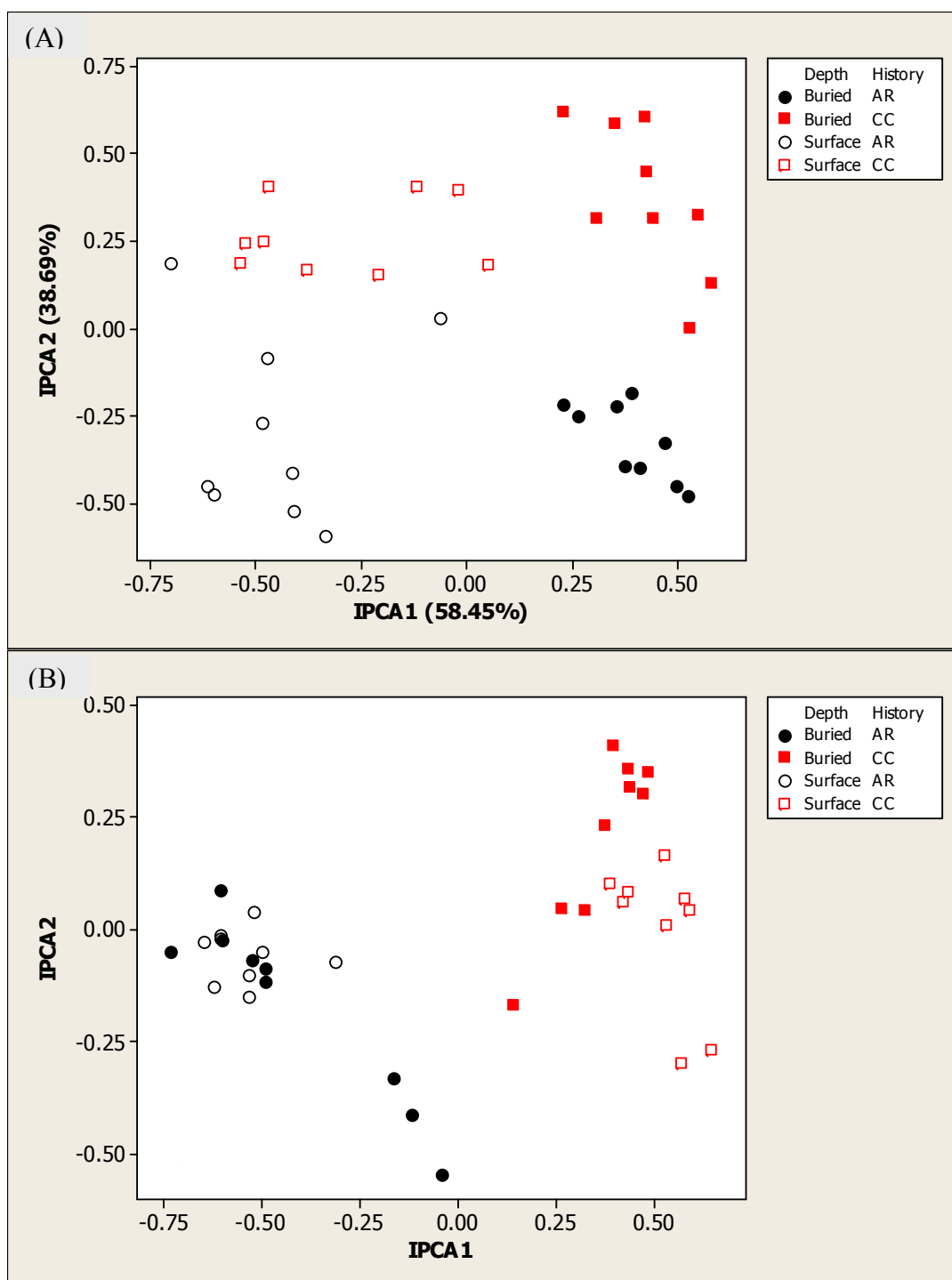


Figure 2.6 Fungal communities colonizing residues in litterbags, HhaI restriction enzyme, labeled by depth and plot history in (A) 2005; and (B) 2006.



## 2.4 Discussion

### 2.4.1 Effect of Treatment (Bt, NonBt and NonBt+I) on Lignin Concentration and Residue Decomposition Rate

Lignin is a major structural component of plant tissue and is decomposed more slowly by microorganisms than cellulose or hemicellulose. A high lignin concentration in crop residues may lead to slower overall rates of residue decomposition and CO<sub>2</sub> evolution (Castaldini *et al.*, 2005). For Bt corn, there is no obvious mechanism that would explain a change in plant lignin concentration resulting from transgene insertion. The gene transformed into Bt corn plants (*cry3Bb1*) is not part of the lignin biosynthesis pathway (Jung and Sheaffer, 2004), however, it is theoretically possible to have pleiotropic effects resulting from the transformation event (Snow *et al.*, 2004). In this study, there was no significant effect of treatment (Bt, NonBt and NonBt+I) on initial lignin concentration or on the rate of litter decomposition. This result is consistent with the findings of Lehman *et al.* (2008) who buried litterbags containing residues (stalks+leaves) from Cry3Bb, Cry1Ab, stacked Cry1Ab/Cry3Bb, and NonBt control corn in a wheat field for 22 months. No differences in decomposition rate or lignin concentrations were found.

The results of this study are also in accord with a laboratory study conducted by Hopkins and Gregorich (2003) and field experiments conducted by Jung and Sheaffer (2004), Zwahlen *et al.* (2007), and Tarkalson *et al.* (2008), where Cry1Ab Bt corn was tested. However, a higher lignin concentration of Cry1Ab Bt corn and slower rates of microbial respiration in response to adding finely ground residues to soil were obtained in a several reports (Saxena and Stotzky, 2001; Castaldini *et al.*, 2005; Flores *et al.*, 2005; Fang *et al.*, 2007). Poerschmann *et al.* (2005) studied the molecular composition of lignin in Cry1Ab Bt corn by tetramethylammonium hydroxide (TMAH)

treatment combined with gas chromatography–mass spectrometry (GC–MS). They found that the total lignin concentration was higher for Bt stalks compared to NonBt stalks, but there was no significant difference between Bt and NonBt leaves. Escher *et al.* (2000) measured the lignin concentration during decomposition of soaked leaves incubated in plastic boxes without soil. They found that Cry1Ab Bt corn (X4334-EPR) leaves had a lower lignin concentration between 2 and 4 weeks after the start of decomposition.

Different lignin detection methods were used in the various studies. For example, the acetyl bromide method was adopted by Saxena and Stotzky (2001) and Flores *et al.* (2005), while ADL was used in this study and those of Lehman *et al.* (2008) and Fang *et al.* (2007). There is no “clear winner” among the different methods for the accuracy of measurement for total lignin concentration in a given sample (Hatfield and Fukushima, 2005). The acetyl bromide method requires a well-defined lignin standard and relies on it to quantify lignin in test samples (Hatfield and Fukushima, 2005). The ADL method may underestimate the total lignin concentration because some of the lignin may be soluble in the acid detergent solution (Shimojo and Goto, 1984; Kondo *et al.*, 1987). However, Jung and Sheaffer (2004) compared the ADL, Klason and acetyl bromide methods for determining lignin concentrations and concluded that the different techniques did not generate different results or alter their conclusions.

The conflicting results obtained by different researchers for lignin concentration of crop residues containing the Cry1Ab protein might result from the different environmental conditions under which these crops were grown. In my study, the lignin concentration of cobs in AR plots was significantly different ( $p < 0.05$ ) from that in CC plots, even though they were grown on the same soil type within the same farm. This result indicates the importance of comparing plants grown under identical

conditions. Another reason why different outcomes were obtained in the different studies may be due to the different hybrids used by different researchers. It is important to point out that variations in lignin concentration and/or decomposition rate also exist between different non-transgenic hybrids, which have not been tested as intensively as TCs. If the lignin concentration of Bt corn is within the same range as that of traditional hybrids, it should not be necessary to test them further. This decision should depend more on the agriculture practices that TCs displace.

The mesh size of litterbags used in this study was 2 x 3 mm (ellipse). This size allowed the microflora (bacteria, actinomycetes, fungi and algae), microfauna (Protozoa, Rotatoria and Tardigrada), and mesofauna (Nematoda, Acari and Apterygota) to enter the litterbags. As the size of some macrofauna (Enchytraeidae, Lumbricidae, Mollusca, Araneae, Isopoda, Myriapoda, Coleoptera and Diptera) is approximately 1-20 mm (Zwahlen *et al.*, 2007), most of these organisms would have been excluded from the litterbags in my study. Hence, any affect that the Bt residues may have on these populations that in turn influences residue decomposition would not be detected. Zwahlen *et al.* (2007) used litterbags with three different mesh sizes (20, 125 and 5000  $\mu\text{m}$ ) and found no significant differences in the litterbag weight remaining for any of the mesh sizes between the Cry1Ab Bt and NonBt corn residues at the end of that experiment.

#### **2.4.2 Effect of Treatment (Bt, NonBt and NonBt+I) on Bacterial and Fungal Communities Colonizing Residues**

Overall, there were no differences between bacterial or fungal communities colonizing the residues from the different treatments (Bt, NonBt and NonBt+I), except in a single assay. In 2006, Hha1 digests of fungal communities colonizing buried residues from the AR plots grouped by treatment. However, no other separation by residue treatment

was found for any other buried or surface-placed residue samples from the CC or AR plots over two years for TRFs derived by use of either the HhaI or MspI restriction enzyme. This study and results from several other studies show that microbial communities are unaffected by TCs. Devare *et al.* (2004) reported that there was no effect of Cry3Bb Bt corn or insecticide application on soil bacterial communities at another NY field site. Icoz *et al.* (2008) investigated the effect of Cry3Bb and Cry1Ab crops on microbial diversity during the process of residue decomposition by use of most probable number (MPN) counts, denaturing gradient gel electrophoresis (DGGE) DNA fingerprints of 16S rRNA gene amplicons and enzyme activity assays. They found that there was no significant effect of Bt as compared to NonBt crops on any of these variables. In my study, an effect of treatment on fungal communities colonizing residues in litterbags was observed in only one of sixteen comparisons. Similarly, in a growth chamber experiment, Blackwood and Buyer (2004) found a significant effect of Bt protein and corn genotype on rhizosphere bacterial communities for only one of three soils tested. They used community-level physiological-profiling (CLPP) to compare communities and found that the amount of variability explained by plant genotype was small, while no significant effect on CLPP was found for the other two soils. Phospholipid fatty acid analysis (PLFA) did not detect any effect of Bt corn on rhizosphere bacterial communities in any of the three soils. They concluded that the effect of Bt corn on microbial communities was not significant. Baumgarte and Tebbe (2005) investigated rhizosphere bacterial community composition using the single-strand conformation polymorphism (SSCP) method and found the effect of Cry1Ab corn was less than that of other environmental factors, such as plant age or field heterogeneities.

### 2.4.3 Plant Part

Plant parts differed significantly in both lignin concentration and decomposition rate. The order of plant parts from highest to lowest lignin concentration was roots > stalks > cobs > leaves; while the order from fastest to slowest decomposition was shoots > cobs > roots. Thus, higher lignin concentration was correlated with a slower decomposition for plant parts. Lignin is more difficult to decompose than cellulose and hemicellulose, the two other major plant structural carbohydrates (Hopkins *et al.*, 2001) and is most likely the reason for observed difference in decomposition rates. This is consistent with the results reported by Fang *et al.* (2007), who also found the order from highest to lowest lignin concentration was roots > stalks > leaves.

In previous studies, stalks, leaves or mixed samples (including all plant parts) were tested to evaluate the effect of transgenic Bt plants on lignin concentration and rate of residue decomposition (Escher *et al.*, 2000; Saxena and Stotzky, 2001; Hopkins and Gregorich, 2003; Jung and Sheaffer, 2004; Castaldini *et al.*, 2005; Flores *et al.*, 2005; Poerschmann *et al.*, 2005; Zwahlen *et al.*, 2007; Lehman *et al.*, 2008). However, only one study included root residues (Fang *et al.*, 2007) and one other included cobs (Tarkalson *et al.*, 2008) to compare with other plant parts. In practice, all roots and some cobs are left in the field along with other plant parts after harvest. Thus, cobs and roots need to be assessed in residue decomposition research. In this study, only the initial lignin concentration for cobs was significantly affected by plot history, indicating the importance of testing different plant parts. Roots had the highest lignin concentration and were the most difficult to decompose, yet neither lignin concentration nor decomposition rate differed between the corn genotypes.

The weight remaining of surface-placed root samples after 3.5 and 15.5 months and some buried root samples after 3.5 months was higher than their initial AFDW.

The most likely explanation is the common limitation of litterbag studies-- encroachment of soil into the litterbags (Moore-Kucera and Dick, 2008). As root litterbags contained initially the smallest sample weight (5 g) and decomposed most slowly, it is possible that soil encroachment could have affected the results. Moreover, when litterbags were collected from the field, new roots were often observed growing into or through the root litterbags, but were rarely seen in cob and shoot litterbags. It is possible that some new root material was retained in the bags when they were taken from the field. The presence of arthropods and their excretions in litterbags might be a source of contaminating materials, but is unlikely to be the main reason for observed gains in litterbag weights over time.

It was surprising that the bacterial and fungi communities colonizing residues did not differ by plant part. The fragment size, surface-to-volume ratio, lignin concentration, C content/composition and fragility differ between plant parts and these attributes may affect decomposer community composition (Burgess *et al.*, 2002). Although roots had a significantly higher lignin concentration compared to other plant parts, microbial communities colonizing the roots did not separate from other plant parts in their T-RFLP fingerprints based on the AMMI analyses. The differences in microbial communities in relation to plant part might be masked by other environmental factors that affected community composition more strongly, such as year, placement or plot history.

#### **2.4.4 Environmental Factors**

The environmental factors associated with year and depth of litterbag placement significantly affected both the decomposition rate and the microbial communities colonizing the residues.

Year was the dominant factor controlling microbial community composition when

data from the two years were analyzed together. Climate factors, such as temperature and precipitation, vary by year and are often the variables found to most strongly influence biological populations. Moreover, litter quantity and quality changed over time during the process of decomposition (Wedin *et al.*, 1995). All of these factors may have caused changes in microbial communities, resulting in inter-seasonal differences.

In this study, all surface-placed residues decomposed slower than the buried samples did and bacterial and fungal community compositions separated clearly by depth. This result is consistent with other studies (Holland and Coleman, 1987; Alva *et al.*, 2002; Burgess *et al.*, 2002) and can be explained by differences in key environmental factors, such as temperature and moisture, that differ dramatically between the two locations. Residues also have less residue-to-soil contact when placed on the soil surface (Summerell and Burgess, 1989) and have more UV exposure, which will also affect microorganisms. Moreover, Holland and Coleman (1987) found microbial activity on buried residues was significantly higher than surface-placed residues, as estimated by uptake of  $^{14}\text{C}$  derived from labeled materials.

The host range of CRW larvae includes some grass species other than corn (Branson and Ortman, 1967, 1970; Clark and Hibbard, 2004), but no feral western CRW has been found to survive without maize (Wilson and Hibbard, 2004). For this reason, the AR plots were assumed to have little or no CRW pressure in the first year, but CRW numbers would likely increase in the following years.

The 2006 surface-placed shoot samples, 2007 buried samples and 2007 surface-placed shoots from CC plots decomposed significantly faster than corresponding samples from AR plots. This is likely due to the microbial communities being pre-adapted to decompose corn residues in the CC plots. The T-RFLP data generated in

this study also indicated that plot history was a dominant factor controlling the composition of the fungal community colonizing residues in 2006.

The plots with the two different cropping histories were in different locations within the same farm. Although the soil type was the same and the plots received the same management, the land use history led to different soil conditions including the soil biota. In 2006, Cortet *et al.* studied the cropping system effects of Cry1Ab Bt and NonBt corn on wheat straw decomposition in fields under three different European climatic conditions. They found that growing a Bt corn crop did not affect wheat residue decomposition, which was mainly affected by climatic conditions, such as temperature, similar to what I observed in this study.

## **2.5 Conclusions**

There was no significant effect of treatment (Bt, NonBt and NonBt+I) on initial residue lignin concentration, residue decomposition rate or bacterial communities colonizing the residues in litterbags. The effect of treatment on fungal communities colonizing the residues in litterbags was minor, with only one comparison showing separation by treatment (2006 buried samples from AR plots for HhaI digests).

Residue lignin concentration and decomposition rate differed significantly by plant part, but microbial communities colonizing these residues did not differ for the different plant parts. The order of plant parts from highest to lowest lignin concentration was roots > stalks > cobs > leaves; while the order from fastest to slowest decomposition was shoots (stalks + leaves) > cobs > roots.

Environmental factors, including year, litterbag placement and plot history, led to significant differences for most variables measured in this study. Microbial communities colonizing the residues in litterbags differed clearly by year. Buried litter



samples decomposed significantly faster than surface-placed residues and their microbial communities were clearly different. Plot history had significant effects on lignin concentration and decomposition rate for some samples and microbial communities colonizing corn residues differed clearly in relation to this factor.

Year was the dominant factor in shaping the microbial community when the two years' data were analyzed together. When analyzing each season separately, depth was the dominant factor in shaping 2005 bacterial and fungal community composition and 2005 bacterial community composition, while plot history was the dominant factor influencing 2006 fungal community composition.

Combined, these results indicate that differences in measured variables were driven primarily by environmental factors rather than by any inherent differences between the corn hybrids (genotypes) or use of the insecticide tefluthrin. In short, the Cry3Bb corn tested in this study did not affect residue decomposition, thus is unlikely to affect C turnover in soil. Since global climate change is linked strongly to emission of greenhouse gases to the atmosphere, these results suggests that Cry3Bb corn will not have any significant effects on global climate change with respect to changes in CO<sub>2</sub> emitted during residue decomposition.

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### **CHAPTER 3**

#### **DEGRADATION OF CRY3Bb BT PROTEIN IN TRANSGENIC CORN RESIDUES IN THE FIELD OVER THREE YEARS**

##### **Abstract**

Reducing tillage retains more residues in the field and is a practice thought widely to have environmental and economic benefits. As the land area planted to Bt crops continues to increase and more residues remain in the field where reduced tillage management has been adopted, the quantity of Bt protein remaining in the field will be significantly greater than experienced previously with the use of Bt sprays. Thus, it is important to investigate the persistence of Bt protein in these residues and evaluate the potential for effects on non-target organisms. In this study, the concentration of Cry3Bb protein in corn residues placed in litterbags was measured over three years of decomposition in a field planted to corn to investigate the dynamics of protein degradation. A double-antibody sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) and PathoScreen kit (Agdia, Elkhart, IN) was used to measure the Cry3Bb protein concentration in Bt corn residues used to prepare the litterbags and in residues recovered from the litterbags after 3.5 and 15.5 months in the field. The Cry3Bb protein had decomposed nearly completely in the plant tissues recovered from the litterbags after 3.5 months in the field. The concentration of Cry3Bb protein differed between the different plant parts. The concentration in root residues was negligible, with only  $0.1 \text{ ng g}^{-1}$  tissue on average, despite the fact that Cry3Bb Bt corn was developed to suppress the corn rootworm (CRW). The rapid disappearance of the Cry3Bb protein from the residues suggests that the protein is unlikely to pose any significant ecological risks to soil organisms. The low concentration of Cry3Bb protein measured in the roots at harvest may not reflect accurately that produced

during active growth, but if low expression during early growth is the norm, this may have implications for the economic effectiveness of Cry3Bb crops and the development of insect resistance.

### **3.1 Introduction**

*Bacillus thuringiensis* (Bt) is a Gram-positive soil bacterium that produces parasporal crystalline inclusions during sporulation. Proteins in these inclusions have a “highly specific insecticidal activity” (Hofte and Whiteley, 1989). Bt whole cell formulations have been used widely as biological insecticides in agriculture to control target pests for more than five decades (Adang, 1991) and are approved for use on organic farms. Crops that are engineered to express the Cry protein gene from Bt (Bt crops) were first planted in the U.S. in 1996 (James, 2006). Since then, Bt crops have been adopted rapidly by farmers and accounted for about 18% of the global area planted to transgenic crops (TCs), which reached 20.3 million ha in 2007 (James, 2007). If TCs with stacked traits (e.g., insect resistance and herbicide tolerance) are included, the percent area planted to TCs expressing the Bt protein gene is even higher. Bt crops can help to reduce the conventional practice of spraying broad-spectrum synthetic insecticides on crops, which has serious detrimental effects on human health and the environment (Ando and Khanna, 2000).

Reduced tillage is thought to have the potential to sequester carbon (C) on a large-scale (Bernacchi et al., 2005), reduce erosion and runoff (Uri, 2000) and save farmers time and money (Service, 2007). Derpsch (2005) reported that no-tillage “is showing increasing interest by farmers”. In this agriculture management system, more plant residues are left in the field. As the land area planted to Bt crops continues to increase and more residues are left in the field, the environmental fate of the Bt protein in residues and its potential ecological impacts remain of interest.



The possible routes for Bt proteins to enter the soil include root exudates (Saxena *et al.*, 1999), pollen (Losey *et al.*, 1999), natural wounding, senescence of root cells (Susanne and Christoph, 2005), or decomposition of plant residues (Tapp and Stotzky, 1998). The persistence of Bt proteins in soil will depend on the crop species and variety, soil type, climate factors and the structure of the protein expressed in plant tissues.

The Cry1Ab protein, active against the Lepidoptera, was observed to bind tightly to surface-active particles in soil (clays and humic acids), which retarded its biodegradation and increased its persistence in soil (Saxena *et al.*, 1999), while its' insecticidal activity was retained (Tapp and Stotzky, 1998). Increased concentration of Bt protein in soil, over that occurring naturally or resulting from the use of Bt sprays, may potentially affect non-target organisms and microbially-mediated soil processes (Icoz and Stotzky, 2008). However, Dubelman *et al.* (2005) did not detect the persistence or accumulation of Cry1Ab protein in soils with clay contents ranging from 11 to 25% in a field planted with Bt corn for at least three consecutive years.

Hopkins and Gregorich (2005) found that the Cry1Ab protein from post-harvest Bt corn residues decomposed rapidly in a laboratory study. They did not detect the protein after incubating the residues for 14 days in a soil with 39, 27, and 34% sand, silt and clay, respectively. Zwahlen *et al.* (2003) found a rapid initial decay followed by slow degradation of Cry1Ab protein in transgenic corn leaves contained in litterbags buried in two fields managed with and without tillage. Bt protein could still be detected at the end of the experiment after 200 and 240 days in two fields, although the amount of plant residue remaining in the litterbags was quite small by that time. The soil used in their study was a Cambisol loam (50, 33 and 17% sand, silt, and clay, respectively).

Crop types were observed to affect the Bt protein released in root exudates (Saxena *et al.*, 2004). Saxena *et al.* (2004) found that the exudates of Cry1Ab Bt corn, potato and rice contained the Bt protein, but those of Cry1Ac Bt cotton, canola and tobacco did not. Head *et al.* (2002) did not detect any Cry1Ac protein in soil samples collected 3 months after the last seasons' tillage from within and outside six fields planted with Bt cotton for 3-6 consecutive years.

Icoz and Stotzky (2007) conducted a laboratory study and found that Cry3Bb1 protein, active against the Coleoptera, degraded rapidly and did not persist or accumulate in the soils they tested. However, they suggested further studies were needed since soils differ in their physicochemical and biological characteristics and these are likely to be important factors determining the persistence of the protein in different soils and locations. No Cry3Bb1 protein was detected in soil planted with Bt corn for one or three consecutive years near Manhattan, KS (Ahmad *et al.*, 2005). However, in another location near Scandia, KS with one-year of Bt corn planting history, the Cry3Bb1 protein was detected at a level of 3.38 - 6.89 ng g<sup>-1</sup> dry soil during the growing season in soil samples collected from areas near the base of the plants, while no protein was detected in soil samples collected from between the rows. The extraction efficiency was found to be lower for the Manhattan than for Scandia soil (Ahmad *et al.*, 2005). This could be explained by differences in the clay content of the Manhattan (36%) and Scandia soils (5%).

Although the decomposition of plant residues is important for monitoring the environmental fate of Bt protein, no study has assessed the dynamics of Cry3Bb protein degradation in plant residues remaining in the field after harvest. This study was conducted to determine the rate of decomposition of the Cry3Bb protein in corn residues in litterbags over two years in a corn field.

## **3.2 Materials and Methods**

### **3.2.1 Field Plots**

Field trials were established at the Cornell University's Musgrave Farm in Aurora, NY, and continued for 3 consecutive seasons from 2004 to 2006. The main treatments were: (1) MON863 corn producing the Cry3Bb protein (Bt); (2) a non-transgenic isoline (NonBt), and (3) the NonBt isoline with tefluthrin insecticide Force 3G applied at planting (NonBt+I). Only the Bt and NonBt treatments were examined in this study. The field soil is a Lima loam: 43.8% sand, 37.4% silt, 19% clay, with a pH of 7.4, and 4.6% organic matter. Seeds of Bt corn (MON863) and its NonBt isoline were obtained from Monsanto Corp. (St. Louis, MO).

The treatments were established in plots rotated out of alfalfa (AR) and in plots continuously planted to corn (CC) within the same farm. Prior to the initial planting of this experiment, the CC plots were found to have corn root worm (CRW) present in sufficient numbers to damage corn roots (Leslie Allee, Entomology, Cornell University, pers. comm.), while the AR plots had little or no CRW evident in the first year after planting.

Each treatment had three replicate plots (50 x 50 m). A randomized complete block (RCB) design was used. Blocks established in the AR plots were contiguous. One CC block was adjacent to the AR blocks and the other two were within 200 m.

### **3.2.2 Residues and Litterbags**

Corn cobs, shoots (stalks + leaves) and roots were collected separately from the field after harvesting in 2004 and stored at 4°C. These residues were oven-dried (65°C) to a constant weight and then used to prepare the litterbags. The starting concentration of the Cry3Bb protein in the plant tissues was measured in subsamples that were ground

in an ED-5 Thomas Wiley mill grinder (Arthur H. Thomas, Co. PA) to a sieve size of 2 mm.

Separate mesh bags (12.5 x 12.5 cm; 2 x 3 mm mesh size ellipse) were filled with 7 g of cobs, 10 g of shoots or 5 g of roots with two duplicate bags for each retrieval time point. In litterbags containing shoots, the ratio of stalks to leaves was 3:2 and was determined by measuring the ratio of these two components in collected field subsamples. Litterbags were placed on the soil surface or at 10 cm depth in the field on June 24, 2005. Each type of residue was placed in plots in which the parent genotype was grown. Each spring during tillage and each fall during harvesting, the litterbags were removed from the field and stored at 4°C for about 2 weeks until those activities were completed and then the litterbags were returned to their respective plots. Litterbags were sampled on October 10, 2005, October 19, 2006 and August 3, 2007 after 3.5, 15.5 and 25 months in the field, respectively. The Bt protein content of residues in the litterbags was measured for the first two retrieval times.

In 2005, the sampled litterbags were taken to the New York State Agricultural Experiment Station in Geneva, NY, where arthropods were extracted using the Tullgren funnel method (data not shown). After arthropods were extracted, the litter samples were dried to a constant weight in a 65°C oven and then ground prior to measuring the Cry3Bb Bt protein concentration in the tissues.

In 2006, the Cry3Bb protein concentration was measured in residues remaining in the litterbags without any pretreatment, wherever possible. Soil intruded into the litterbags for the buried root samples. Root material was separated from soil using a particulate organic matter (POM) extraction (Christensen, 1992). A series of 12.7 cm (5-inch) diam nesting sieves were stacked in the order of 2 mm, 250 µm, 53 µm mesh size from top to bottom. Litterbag residues were placed on the top sieve and then

rinsed through the next two sieves with water. Materials remaining in all three sieves were transferred to a container and then recovered by a density gradient fractionation. The cleaned root residues were placed in a 65°C oven for one week and then ground for Bt protein analysis.

### **3.2.3 Cry3Bb Protein Detection**

A double-antibody sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) and PathoScreen kit (Agdia, Elkhart, IN) was used to determine the Cry3Bb protein concentration in Bt corn residues recovered from the litterbags after 3.5 and 15.5 months in the field.

Duplicate 0.1 g ground corn materials were sub-sampled from the 2004 field residues and residues recovered after 3.5 months in the field (2005) and 15.5 months in the field (2006). Subsamples were placed into 2 ml Eppendorf tubes with 1 ml of 1x PBST (phosphate buffered saline plus 0.07% (v/v) Tween-20) extraction buffer. The tubes were vortexed for 15 s and then centrifuged at 3500 x g for 20 min. A 1 ml aliquot of the supernatant was transferred into a new tube and centrifuged again at 5000 x g for 5 min. Following the instructions provided in the Agdia PathoScreen kit, 100 µl of the supernatant was added into the test wells of the Agdia ELISA plate along with 100 µl of peroxidase enzyme-conjugate and incubated in a humid box for 2 h at room temperature. Then the test wells were emptied and washed with 1x PBST six to eight times. A 100 µl aliquot of tetramethyl benzidine (TMB) substrate solution was dispensed into each empty test well and incubated in a humid box again for 20 min. The ELISA plate was analyzed by a V<sub>max</sub> enzyme kinetic microplate reader (uQuant; Bio-Tek Instruments, Inc., Winooski, VT) to determine the optical density at 635 nm. A five-point standard curve was prepared using purified Cry3Bb protein provided by Monsanto Co. (St. Louis, MO) and used to calculate the protein concentration in

sampled tissues. For samples with high concentrations of the protein, dilutions were made so that readings were within the linear range of the Cry3Bb protein standard curve.

### 3.3 Results

For the residues used to prepare the litterbags, different plant parts had significantly different Cry3Bb protein concentrations ( $p=0.0001$ ) (Table 3.1). Leaves had the highest Cry3Bb protein concentration, with  $124.2 (\pm 36.7) \text{ ng g}^{-1} \text{ tissue } (\pm \text{SE})$ . The Cry3Bb protein concentrations in stalks and cobs were similar,  $20.9 (\pm 5.2)$  and  $19.8 (\pm 8.5) \text{ ng g}^{-1} \text{ tissue}$ , respectively. In roots, the Cry3Bb protein concentration was negligible, only  $0.1 \text{ ng g}^{-1} \text{ tissue}$  on average. There was a significant interaction between plant part and plot history ( $p=0.01$ ) for the initial Cry3Bb protein concentration in residues (Table 3.1). The protein concentration in Bt corn leaves from

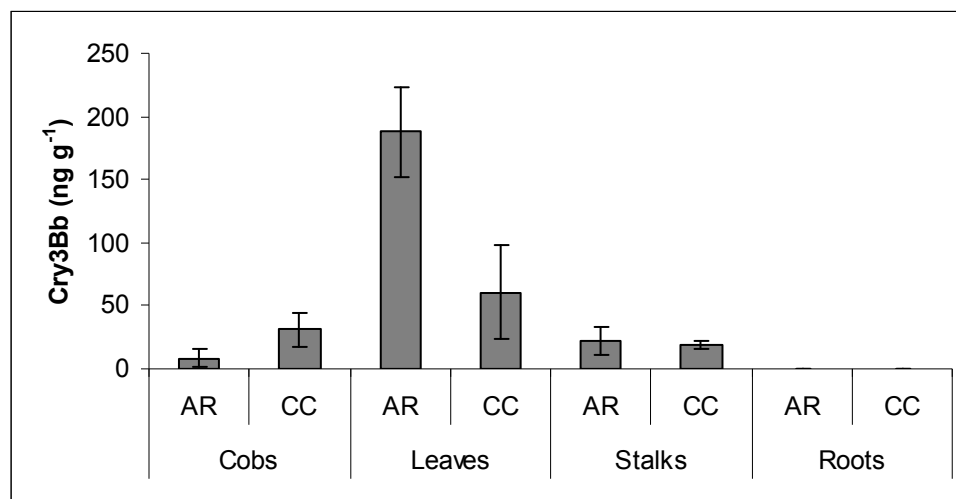


Figure 3.1 Cry3Bb protein concentrations in the residues of different plant parts at the start of the residue decomposition experiment (error bars: SE).

Table 3.1 ANOVA for Cry3Bb protein concentration in 2004 corn residues.

Source of Variance	Cry3Bb Protein Concentration		
	df	F-value	p-value
Plant part (P)	3	16.57	<b>0.0001</b>
Plot history (H)	1	3.82	0.07
P × H	3	6.04	<b>0.01</b>

AR plots was significantly higher ( $p < 0.05$ ) than the concentration in leaves from CC plots. However, the Cry3Bb protein concentration in cobs from CC plots was higher than that in cobs from AR plots (Figure 3.1).

After 3.5 months of decomposition in the field, the Cry3Bb protein had decomposed nearly completely in the residues recovered from the litterbags (Table 3.2). The average concentration of Cry3Bb protein in all residues retrieved in 2005 (3.5 months) and 2006 (15.5 months) was only 0.2-0.3 ng g<sup>-1</sup> tissue. For litterbags sampled after 3.5 months, excluding the root data, only plant part was a significant factor ( $p = 0.05$ ) for the percentage of Cry3Bb protein content remaining in decomposing plant tissues (Table 3.2). On average, 5.22% of the Cry3Bb protein was still detectable in cobs, while only 0.47% was still detectable in stalks+leaves. For the litterbags retrieved after 15.5 months, depth of residue placement was a significant factor ( $p = 0.05$ ) (Table 3.2). On average, 2.88% of the starting Cry3Bb protein content remained in residues contained in surface-placed litterbags, while only 0.27% of the starting Cry3Bb protein content remained in residues contained in buried litterbags.

The starting Cry3Bb protein concentration in root residues was negligible. However, for root residue litterbags, the average concentration in tissues recovered after 3.5 and 15.5 months in the field was 0.3 ng g<sup>-1</sup>. In essence, there was no change in the protein concentration in these tissues over time, which was close to the limit of detection for the assay.

### 3.4 Discussion

After 3.5 months in the field, virtually all of the Cry3Bb protein tested in corn residues was decomposed. This is consistent with the results of Icoz and Stotzky (2007), who found that the Cry3Bb1 protein degraded rapidly in soil during laboratory incubations. They placed a mixture of Bt corn (roots, stems and leaves) in an unamended

Table 3.2 Percentage of Cry3Bb protein content in residues remaining in litterbags recovered after 3.5 and 15.5 months in the field.

Plant Part	Litterbag Placement	Plot History	Cry 3Bb Protein After 3.5 Months % ( $\pm$ SE)	Cry3Bb Protein After 15.5 Months % ( $\pm$ SE)		
Cobs	Buried	AR	13.56 ( $\pm$ 12.76)	1.03 ( $\pm$ 0.91)		
		CC	1.51 ( $\pm$ 0.76)	0.17 ( $\pm$ 0.07)		
	Surface	AR	8.07 ( $\pm$ 6.89)	7.65 ( $\pm$ 6.12)		
		CC	1.50 ( $\pm$ 0.71)	3.39 ( $\pm$ 2.08)		
Shoots	Buried	AR	0.26 ( $\pm$ 0.02)	0.03 ( $\pm$ 0.00)		
		CC	0.67 ( $\pm$ 0.31)	0.09 ( $\pm$ 0.06)		
	Surface	AR	0.21 ( $\pm$ 0.06)	0.30 ( $\pm$ 0.13)		
		CC	0.91 ( $\pm$ 0.26)	0.90 ( $\pm$ 0.06)		
Source of Variance		df	F-value	p-value	F-value	p-value
Depth (D)		1	0.20	0.66	<b>4.72</b>	<b>0.05</b>
Plant part (P)		1	<b>4.88</b>	<b>0.05</b>	3.84	0.08
Plot history (H)		1	2.07	0.18	0.73	0.41
D $\times$ P		1	0.26	0.62	2.61	0.13
D $\times$ H		1	0.22	0.65	0.28	0.61
P $\times$ H		1	4.27	0.06	1.30	0.28
D $\times$ P $\times$ H		1	0.34	0.57	0.60	0.46

Kitchawan soil (57% sand, 34% silt, 9% clay) or soil amended with 3 or 6% (v/v) montmorillonite or kaolinite and incubated the soils at 25 ( $\pm$  2) $^{\circ}$ C at –33 kPa water tension for 60 d. Although the physicochemical and biological characteristics of the soil might be important for persistence of the protein, no protein was detected after 40 d incubation in any of the soils or amendments they tested. The rapid decomposition of Cry3Bb protein indicates that it likely poses little ecological or environmental risk



to soil biotic communities. However, it is possible that some of the Cry3Bb protein leached from the residues in litterbags and continued to persist in the soil. Further study is needed to test whether the protein was degraded by decomposers or passed into the soil with water movement through the litterbags.

Different plant parts contained different concentrations of Cry3Bb protein. The order for Cry3Bb protein concentrations from highest to lowest was leaves > stalks = cobs > roots. Roots contained only 0.1 ng g<sup>-1</sup> tissue on average. Results reported in the USEPA (2003) MON863 registration document show that roots could have a low Cry3Bb1 protein concentration, ranging from 3.2 to 66 µg g<sup>-1</sup> fresh tissue. Our data agree with results reported by Wander and Gunapala (2004) where the Cry3Bb protein concentration in tissues also decreased in the order of leaves > stems > roots. As Cry3Bb Bt corn was developed to suppress the corn rootworm which feeds on corn roots, the low protein expression in the roots may have both ecological and economic implications. If Bt protein expression in roots is low, the TC may be ineffective at controlling a severe CRW infestation and could result in significant economic losses for farmers. Moreover, low expression may hasten the evolution of pest resistance (Tabashnik, 1994). However, the residues in this study were collected from the field after harvest and thus, these concentrations may not represent the expression level in the early stages of corn growth when CRW root feeding is likely to be most severe. Further study is needed to establish the profile of protein expression in different tissues across the crop growth cycle and under varying field conditions.

There was as significant interaction between plant part and plot history for the Cry3Bb protein content of residue tissues (Figure 3.1). Clark *et al.* (2004) reported that there were a very limited number of publications that addressed changes in Bt protein expression under different edaphic or climatic conditions. They assumed that the tissue protein concentration would be affected by environmental factors. In my

study, plots with different cropping histories were not contiguous, but were within the same farm, on the same soil type, and under the same agricultural management during the experiment. Prior cropping history creates legacy effects (Buckley and Schmidt, 2001) and these were sufficient to result in differences in tissue protein content in the Bt corn grown in this experiment. This is an important point to consider, as researchers seldom provide information about how the residues being tested were grown. If they are obtained from different farms, or different locations within a farm, they may start out having different compositions that may then confound the results of decomposition studies using these residues.

In this study, the average starting Cry3Bb protein concentration in leaves, stalks, cobs and roots were  $124.2 (\pm 36.7)$ ,  $20.9 (\pm 5.2)$ ,  $9.8 (\pm 8.5)$  and  $0.1 \text{ ng g}^{-1}$  tissue ( $\pm$  SE), respectively. These concentrations were quite low compared values reported in other studies. Based on the data submitted to the EPA during the product registration phase, Cry3Bb (MON863) concentration ranges in fresh tissue were  $30\text{-}93 \text{ }\mu\text{g g}^{-1}$  (leaf),  $49\text{-}86 \text{ }\mu\text{g g}^{-1}$  (grain),  $30\text{-}93 \text{ }\mu\text{g g}^{-1}$  (pollen),  $3.2\text{-}66 \text{ }\mu\text{g g}^{-1}$  (root), and  $13\text{-}54 \text{ }\mu\text{g g}^{-1}$  (total above ground plant tissue) (USEPA, 2003). Vaughn *et al.* (2005) reported that the average protein concentration in the roots of five MON863 hybrids was  $69.8 \text{ }\mu\text{g g}^{-1}$  at V4 and  $44.0 \text{ }\mu\text{g g}^{-1}$  fresh tissue at V9. Although all the results reported above were based on fresh weight of plant tissue and my results were based on dry weight, these concentrations are considerably higher than those I measured in this study. The highest tissue concentration found in my samples was from leaves ( $0.12 \text{ }\mu\text{g g}^{-1}$  dry weight), which would be even smaller if expressed on a  $\text{g}^{-1}$  of fresh weight basis and is much lower than the ranges reported to the USEPA (2003) and Vaughn *et al.* (2005).

Icoz and Stotzky (2008) measured the Cry3Bb1 protein in MON863 Bt corn after 28 days of growth in hydroponic culture. They found that the concentration of Cry3Bb1 protein in mixed biomass containing roots, stems and leaves was  $1.6 (\pm 0.20)$

$\mu\text{g g}^{-1}$  dry weight tissue, which was comparable to, but still higher than the protein concentration in leaves measured in my study ( $0.12 \pm 0.04 \mu\text{g g}^{-1}$  dry weight of tissue). Similarly, Wander and Gunapala (2004) found that the concentrations of Cry3Bb1 protein ranged from 1.7 to 2.5  $\mu\text{g g}^{-1}$  fresh weight in leaves, 0.4–1.0  $\mu\text{g g}^{-1}$  fresh weight in stems and 0.1–0.4  $\mu\text{g g}^{-1}$  fresh weight in roots of field grown plants. The reasons for these discrepancies are not known, but are likely due to variations in soil type, nutrient availability, climate and hybrid, among other factors. Clark *et al.* (2005) pointed out that Cry protein gene expression varies in different crops and events because “it is difficult to control the location of insertion of the Bt gene”. The inserted transgenes may interrupt native genes or their promoters, cause small-scale rearrangements (Windels *et al.* 2001), have unanticipated interactions between transgenes and native genes, increase mutations, or affect multiple traits (Snow *et al.*, 2005), including the expression level of Cry protein. Moreover, the different promoters used to control gene expression may cause differences in tissue protein levels over time and between plant parts (Clark *et al.*, 2005).

### 3.5 Conclusions

Residues of field grown Bt corn used to prepare litterbags for use in these decomposition experiments had different concentrations of Cry3Bb protein in the different plant parts and for some residues due to plot history. The order for Cry3Bb protein concentration in corn tissues from highest to lowest was leaves > stalks=cobs > roots. There was a significant interaction between plant part and plot history for the concentration of Cry3Bb protein in corn tissues. After 3.5 months of decomposition in the field, the Cry3Bb protein was nearly non-detectable in all tissues. The rapid disappearance of the Cry3Bb protein suggests that it is unlikely to pose any significant short or long-term ecological risks. As the environmental exposure to Cry3Bb protein

is likely to be limited, non-target organisms are not likely to be affected by the presence of Bt residues in the field. The low concentration of Cry3Bb protein in the roots may have economic implications related to effectiveness of CRW control and insect resistance development if the concentration is also low during early growth.

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## CHAPTER 4

### GROWTH AND CARBON ALLOCATION OF CRY3Bb BT AND NONBT CORN HYBRIDS IN THE GREENHOUSE

#### **Abstract**

Differences in carbon (C) allocation between transgenic plants and non-transgenic parental varieties may affect C turnover and/or sequestration in soil. Three greenhouse experiments were conducted to (a) compare the growth rate, total C content,  $^{13}\text{C}$  allocation, lignin content and concentration of Cry3Bb Bt and NonBt corn as affected by the presence of corn rootworm (CRW); and (b) determine whether differences in plant biomass between Bt and NonBt corn are greater or less than differences between corn plants in different maturity groups. A  $^{13}\text{CO}_2$  pulse-labeling system was used at the V7 stage in Experiment 1 and the R2 –R4 stages in Experiment 2. The  $^{13}\text{C}$  content was measured at the V7 and R5 stages to investigate  $^{13}\text{C}$  dilution with time in Experiment 1 and at the R5 stage to compare  $^{13}\text{C}$  allocation in Bt and NonBt corn as affected by CRW presence in Experiment 2. Lignin content and concentration were determined by the acid detergent lignin (ADL) method. In Experiment 3, three maturity groups (MGs) of Bt and NonBt corn were planted to compare the variance between and within MGs. There were no significant effects of genotype (Bt or NonBt) on total C content,  $^{13}\text{C}$  allocation or lignin concentration or content in corn tissues. These results suggest that the Bt transgenic event in MON863 corn is unlikely to change soil C budgets significantly. However, CRW inoculation resulted in higher total C content, lignin concentration and plant height in infested plants, including both Bt and NonBt hybrids. Induced systemic resistance is suggested as an explanation for these observations. Lignin content measured in NonBt roots inoculated with CRW was significantly higher than in inoculated Bt roots. This suggests that the protection from

CRW feeding provided by the expression of the Bt gene, rather than the transformation, was responsible for the differences between Bt and NonBt corn. NonBt corn was significantly taller than Bt corn at the V7, R5 and R7 stages of development, but this is likely a result of normal varietal variation as suggested by the results of the experiment in which variation between three MGs and between Bt and NonBt corn plants within each MG was compared. Aboveground biomass of the 96 d MG was higher than that of the 101 and 111 d MGs ( $p = 0.06$ ). However, no significant difference was observed between the Bt and NonBt hybrids within the 96 d MG, indicating that the difference between Bt and NonBt within that 96 d MG was less than differences between the MGs. Variables measured for Bt corn in this study were within the same range as those for NonBt corn. Thus, if values for Bt corn are within the typical range for NonBt corn, it may not be necessary to exhaustively test these variables for TCs further.

#### **4.1 Introduction**

Transgenic insect resistant (IR) plants, primarily those expressing a crystal (Cry) protein gene from *Bacillus thuringiensis* (Bt crops) (Brookes and Barfoot, 2006), were commercially released in the United States and other countries in the late 1990s (James, 1997; Gould 1998). Bt crops are engineered to produce a Cry protein with insecticidal activity against Lepidopteran, Dipteran, or Coleopteran larvae (Crickmore *et al.*, 1998; Schnepf *et al.*, 1998). Bt crops accounted for about 18% of the global area planted to TCs, which reached 20.3 million ha in 2007 (James, 2007). Bt corn variety MON863, that expresses the Cry3Bb protein with insecticidal activity against the western corn rootworm (CRW), was released for commercial use in the U.S. in 2003 (USEPA, 2003) and is the subject of this study.

Slower rates of residue decomposition of transgenic crops (TCs), as measured by

soil respiration, have been reported by Castaldini *et al.* (2005) and Flores *et al.* (2005). Difference in residue turnover may influence nutrient cycling, greenhouse gas (GHG) emissions and carbon (C) sequestration in soils. The amount of C stored in soils depends on the C balance between inputs from plant and animal residues and CO<sub>2</sub> emissions resulting from the decomposition of residues by microorganisms. Increased C sequestration in soil is one way to reduce atmospheric concentrations of CO<sub>2</sub>, an important GHG. Mulching with crop residues can increase C sequestration in soil for a variety of crops and environments (Aulakh *et al.*, 2001; Bremer *et al.*, 2002; Jacinthe *et al.*, 2002). Carbon turnover and the associated ability to sequester C in soil depend on the quality and quantity of the residues added, their management in the field, soil properties, and environmental conditions, such as moisture and temperature (Duxbury *et al.*, 1989; Follett, 2001).

The carbon balance between C respired and C sequestered in soil depends, in part, on crop residue quality, which may differ between Bt crops and their parental varieties that do not express the *cry* protein gene (NonBt) (Saxena and Stotzky, 2001). Higher lignin content may make residues more difficult for microorganisms to decompose and therefore affect C turnover and sequestration. Conflicting results have been reported for the lignin content of Cry1Ab corn. Saxena and Stotzky (2001) correlated lower respiration from soils mixed with Cry1Ab Bt corn (Bt11, MON810 and Bt176) residues with a 33-97% higher lignin concentration in corn stalks compared with their NonBt isolines. This was confirmed by additional studies in which Cry1Ab Bt corn was tested (Castaldini *et al.*, 2005; Flores *et al.*, 2005; Fang *et al.*, 2007). However, results from other studies show that Cry1Ab corn does not differ from NonBt corn in its lignin concentration or decomposition rate (Hopkins and Gregorich, 2003; Jung and Sheaffer, 2004; Zwahlen *et al.*, 2007; Tarkalson *et al.*, 2008). By using Cry1Ab, Cry3Bb, and stacked Cry1Ab/Cry3Bb Bt hybrids, Lehman *et al.* (2008) also found

that decomposition rates and lignin concentration in transgenic corn and non-transgenic corn did not differ. Most of the studies mentioned above used the term “lignin content”, when they actually reported the lignin concentration (% or g kg<sup>-1</sup>).

C allocation can affect the growth of plants by “shifting the products of photosynthesis between respiration and biomass production, ephemeral and long-lived tissues, and aboveground and belowground components” (Litton *et al.*, 2007). Thus, changes in C allocation can influence C cycling in the ecosystem through effects on litter quality and decomposition rates, C and nitrogen (N) sequestration, and plant–atmosphere gas exchange (Friedlingstein *et al.*, 1999; Bird and Torn, 2006). Variation in C allocation between roots and shoots of Bt vs. NonBt plants can affect C input to soils. C allocation within plants is governed by genotypic considerations such as varietal differences (Vessey, 1992; Percival *et al.*, 2001); physiological factors (Ameziane *et al.*, 1995; Cruz *et al.*, 2001); environmental factors including soil texture, aeration, moisture, pH, and salinity; exposure of roots or shoots to toxic compounds; competition with other plants; and the presence of pathogens or pests (Feldman, 1984; Wild, 1988; Waisel *et al.*, 1991). The latter is especially important in any consideration of the effect of Bt crops on soil C dynamics, protected as these plants are against significant insect damage. A study conducted to elucidate patterns of photosynthesis and C partitioning as a result of above-ground insect damage in cotton showed that yield reduction was correlated with significant differences in C allocation within infested vs. insect-free plants (Holman and Oosterhuis, 1999). Carneiro *et al.* (1999) demonstrated that infection of soybean roots by a nematode significantly increased the amount of C allocated to the roots with an associated decline in C allocation to shoots. Thus, it is reasonable to hypothesize that C partitioning within Bt plants might differ from NonBt isolines in the presence of pest damage. There is some evidence to suggest that plant roots might contribute more to soil C content than

aboveground biomass (Jenkinson, 1971; Puget and Drinkwater, 2001). Therefore, the sequestration of soil C derived from Bt as compared to NonBt corn residues might vary substantially and needs to be examined since Bt corn roots are protected from rootworm damage while those of the NonBt isolate are not.

Altered C allocation and structural C forms, such as higher lignin concentration in different plant tissues, might be responsible for observed differences in decomposition rates between Bt and NonBt residues. In this study, the main goals were to (a) compare the growth rate of Cry3Bb Bt and NonBt corn as affected by the presence of the CRW; (b) measure the C allocated to different plant parts in Cry3Bb Bt and NonBt corn as affected by the presence of the CRW; (c) determine the lignin content and concentration in different parts of the Cry3Bb Bt and NonBt corn as affected by the presence of the CRW; and (d) determine whether plant biomass differences between Bt and NonBt hybrids within each of three maturity groups was greater or less than differences between plants in different maturity groups.

## **4.2 Materials and Methods**

Seeds of MON863 Bt corn that expresses the Cry3Bb protein, active against the corn CRW, and its non-transgenic counterpart were obtained from the Monsanto Co. (St. Louis, MO). Two-gallon plastic pots were filled with steamed Cornell Mix, a growth medium composed of peat, vermiculite and perlite (1:1:1 by volume) with 6 g pulverized limestone, 35 g CaSO<sub>4</sub>, 42 g powdered FeSO<sub>4</sub>, 1 g fritted trace elements (Peters FTE 555, Scotts Co., Marysville, OH), and 3 g wetting agent (AquaGro G, Aquatrols, Pennauken, NJ). Three corn seeds were sown per pot. Each pot was thinned to one corn plant 15 d after sowing. Plants were watered daily and fertilized weekly with a nutrient solution containing 1.08 g of Peter's 15-16-17 L<sup>-1</sup> (Scotts Company). The plants were grown under ambient light from 6:00 to 20:00 h solar time with 400

W high-pressure sodium lights (PAR, 400-700 nm wavelengths). Day and night temperatures averaged 21°C (day) and 13°C (night).

A randomized complete block design was used in all experiments to compare (1) Bt and NonBt corn growth in Experiment 1, (2) Bt and NonBt corn with (+) and without (-) CRW added in Experiment 2, and (3) three maturity groups containing Bt and NonBt genotypes in Experiment 3. Three blocks for Experiment 1 and four blocks for Experiments 2 and 3 were set up in the greenhouse to control for the possible effects of sunshine angles, temperature and humidity on plant growth.

#### **4.2.1 Experiment 1: $^{13}\text{C}$ Allocation in $^{13}\text{CO}_2$ -Labeled Bt and NonBt Plants**

The  $^{13}\text{CO}_2$  pulse-labeling system of Stewart and Metherell (1999) was used to label 18 Bt and NonBt plants from three experimental blocks with 99.99 atom %  $^{13}\text{CO}_2$  when plants were at the V7 stage.

For each labeling, one corn plant was put into a cylindrical Saran gas-proof bag (76 cm diam x 200 cm height; Applied Research Products Ltd., Canada). The bag was sealed to the pot with a rubber band. The pots were sealed at the soil surface with a plastic bag to ensure that the  $^{13}\text{CO}_2$  did not infiltrate the belowground compartment (roots and soil). A pulse of 99.99 atom%  $^{13}\text{CO}_2$  was injected into the chamber at a flow rate of 591 ml min<sup>-1</sup> for 3 to 3.5 min.

Plants were destructively harvested at the V7 stage (2 days after  $^{13}\text{CO}_2$  labeling) and R5 stage (no additional labeling). Plant parts were separated into cobs, husks, leaves, stalks, and roots; except at the V7 stage, when no cobs or husks were present. A 1.0 mg subsample of each ground tissue sample was weighed and sent to the Stable Isotope Facility at the University of California, Davis (Davis, CA) to determine  $^{13}\text{C}$  concentration in the plant tissues by use of a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon

Ltd., Cheshire, UK).

#### **4.2.2 Experiment 2: C Allocation in Bt and NonBt Corn With and Without CRW Inoculation**

At the V6 (vegetative: sixth leaf) stage, the (+)CRW treatments were infested with 100 western corn rootworm (*Diabrotica virgifera virgifera*) eggs per pot. These non-diapausing eggs were obtained from a stock colony maintained at the USDA-ARS Northern Grain Insects Research Laboratory (Brooking, SD). The eggs were stored at 8°C and sent overnight to our laboratory 10 d prior to the target hatch date. The eggs were suspended in 0.125% (w/v) solution of water agar and added to pots by trickling the suspension into three 1.0 cm deep holes (0.008 m<sup>2</sup>) in the surface soil of each pot. The holes were covered with moist soil and these pots were not watered for 24 h. Two extra pots were infested in the same way to monitor egg and larval development (Hou *et al.*, 1997). Five days later, 15 larvae were transferred into the (+)CRW treatment pots because no eggs were observed to hatch in the two extra pots. Larvae were added into a single hole (1 cm diam, 3 cm deep) in each pot using a moist camel hair brush. The hole then was covered with moist soil and these pots were again not watered for 24 h. A fine stainless steel mesh (104 µm) was used to cover the drainage holes of every (+)CRW treatment pot to prevent larvae from escaping (Clark and Hibbard, 2004). Once eggs and larvae were added, these pots were also covered with insect mesh (0.06 mm opening, ECONET L, LS Americas Co., Charlotte, NC) to prevent the escape of CRW adults from these pots. The opening created in the mesh to allow for the growing corn stem was sealed using a cable tie.

Similar to Experiment 1, the <sup>13</sup>CO<sub>2</sub> pulse-labeling system (Stewart and Metherell, 1999) was used to label 48 Bt and NonBt corn with and without CRW twice with 50 atom % <sup>13</sup>CO<sub>2</sub> during the R2 and R4 stages. At each of these labeling times, 0.25 g <sup>13</sup>C

was injected into the chamber for one corn plant.

Plants were destructively harvested at the V7 (vegetative: seventh leaf), R5 (reproductive: dent) and R7 (reproductive: harvest maturity) stages. Plant height was measured before harvest and plants were separated into leaves, stalks, roots, cobs and husks, where present. All plant parts were weighed to obtain fresh weight, thoroughly cleaned with water, blot-dried with paper towels, chopped into small pieces (about 5 cm length) and air-dried. Thereafter, all the harvested plant parts were placed in a forced draft oven set at 57°C until constant weights were obtained. Biomass estimates were based on the oven-dry weight of all plant tissues, which were then ground to pass through a 2 mm mesh Wiley mill grinder (Arthur H. Thomas Co., PA).

Acid Detergent Lignin (ADL) analysis was conducted on stalks, leaves, cobs, husks, and roots following the protocol of Goering and Van Soest (1975). Approximately 1 g of each ground sample was weighed and placed in a 600 mL Pyrex reflux flask. One hundred milliliters of acid detergent solution (composed of 1N sulfuric acid, technical grade cetyl trimethylammonium bromide and 2% decahydronaphthalene) was added to each flask. The flasks were connected to a refluxing apparatus and heated for 1 h. After that, all materials were vacuum-filtered and transferred to Gooch crucibles. The residues were then washed with acetone to remove all color and break up lumps and put into an oven set at 100°C overnight to dry. The acid detergent fiber (ADF) was calculated as follows:

$$ADF = (W_0 - W_t) (100) / S$$

Where:  $W_0$  = weight of oven-dry crucible including fiber

$W_t$  = tare weight of oven-dry crucible

$S$  = oven-dry sample weight

Crucibles containing the ADF were half-filled with cooled (15°C) 72%  $H_2SO_4$  and stirred with a glass rod to break all lumps. The crucible was re-filled with 72%  $H_2SO_4$



three times at hourly intervals and then kept at 20 - 23°C for 3 h. After that, materials were vacuum-filtered and washed with hot distilled water five times to remove the acid. Final filtration was completed by adding acetone to the crucibles and setting them to dry overnight in a 100°C oven. Hot crucibles containing the residues were weighed and put into a 520°C muffle furnace for 3 h, cooled to 100°C and then weighed again. The acid detergent lignin (ADL) was calculated as follows:

$$\text{ADL} = (\text{L} \times 100) / \text{S}$$

Where: L = loss upon ignition after 72% H<sub>2</sub>SO<sub>4</sub> treatment

S = oven-dry sample weight

#### **4.2.3 Experiment 3: Comparison of Above- and Below-Ground Biomass for Three Corn Maturity Groups**

Six hybrids from three maturity groups: (A) 96 d; (B) 101 d; and (C) 111 d to maturity were planted in 4 gallon pots containing a 1:2 mixture of the aforementioned Cornell Mix and pasteurized field soil. Each maturity group (MG) treatment included one Bt and one NonBt corn variety (Table 4.1). At the V5 (vegetative: fifth leaf) stage, plants were destructively harvested and the weight of the different plant parts was measured. After V5, unexpected pests, including fall armyworm and leaf miner infested the greenhouse and this experiment was terminated.

#### **4.2.4 Statistical Analysis**

Analysis of variance (ANOVA) with a linear mixed-effect model was conducted using S-PLUS 8.0 (TIBCO Software Inc., Palo Alto, CA). Block was set as the random factor. All other treatment variables were set as fixed factors. Statistical significance was set at the default level of  $\alpha=0.05$ . For Experiment 3, orthogonal contrasts were used to compare between the different maturity groups and among Bt and NonBt

genotypes within the same MG in an ANOVA analysis.

### **4.3 Results**

#### **4.3.1 Experiment 1: $^{13}\text{C}$ Allocation in $^{13}\text{CO}_2$ -Labeled Bt and NonBt Plants**

Use of  $^{13}\text{CO}_2$  pulse-labeling to determine C allocated to Bt and NonBt plant parts showed that genotype (Bt or NonBt) did not significantly affect the  $^{13}\text{C}$  content measured at the V7 and R5 stage (Table 4.1). However, the  $^{13}\text{C}$  content differed significantly by plant part ( $p < 0.05$ ).

About 53% of the  $^{13}\text{CO}_2$  label accumulated by the corn plants at the V7 stage was lost through respiration and sloughing by the time the plants reached the R5 stage (Figure 4.1). The  $^{13}\text{C}$  content in leaves and stalks at the R5 stage was significantly lower ( $p < 0.05$ ) than that measured 2 days after labeling at the V7 stage, but the  $^{13}\text{C}$  content in roots did not differ significantly between the V7 and R5 stages in t-tests.

#### **4.3.2 Experiment 2: C Allocation in Bt and NonBt Corn With and Without CRW**

NonBt corn was significantly taller than the MON863 Bt corn at all three stages sampled: V7, R5 and R7 ( $p < 0.05$ ) (Table 4.2). NonBt corn was 5.2% taller than Bt corn at the R5 stage. In addition to hybrid, the presence of CRW influenced plant height at the R5 stage ( $p = 0.05$ ) (Table 4.3).

Carbon partitioning measurements conducted at the V7 stage indicated that neither corn hybrid (Bt or NonBt) nor CRW presence had any significant effect on C allocated to different plant parts (Table 4.3). However, the total C content varied significantly by plant part ( $p < 0.0001$ ), with the highest and lowest C content observed in stalks and roots, respectively (Figure 4.2 A).

At the R5 sampling stage, plant part, CRW presence and the interaction between plant part and CRW presence significantly influenced the total C allocated within the corn

plant ( $p < 0.05$ ), while genotype had no significant effect (Table 4.3). In contrast with the C partitioning observed at the V7 stage, at R5, more C was allocated to roots, while cobs and husks had the lowest overall C content (Figure 4.2 B). Adding CRW to pots resulted in a higher total C content on average for all plant parts. Only (+)CRW cobs had a lower total C content when compared to (-)CRW cobs. However, these differences in C allocation did not change the total C root/shoot ratio significantly.

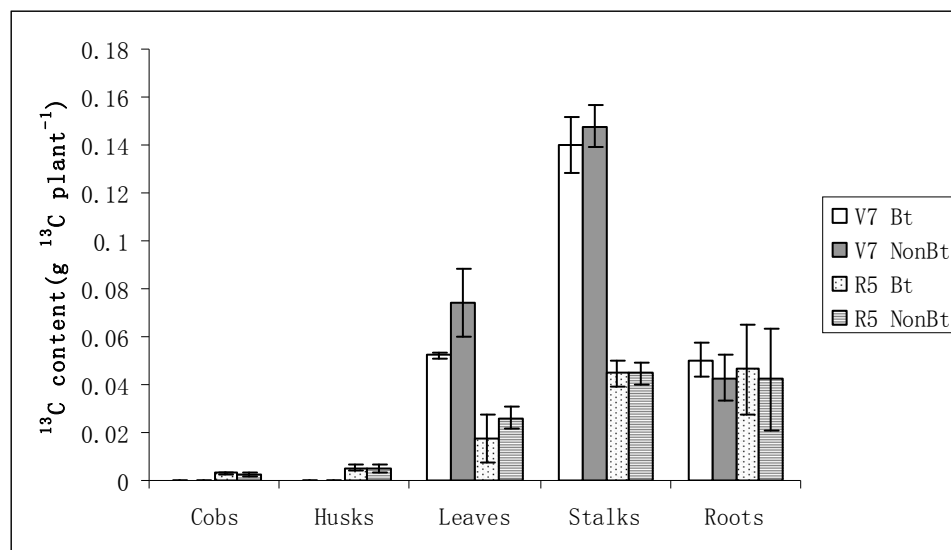


Figure 4.1 Labeled  $^{13}\text{C}$  content in Bt and NonBt corn plant parts at the V7 and R5 stages in Experiment 1 (error bars: SE).

Table 4.1 ANOVA for  $^{13}\text{C}$  content measured at the (A) V7 and (B) R5 stages in Experiment 1.

Source of Variance	V7			R5		
	df	F-value	p-value	df	F-value	p-value
Genotypes (G)	1	1.24	0.29	1	0.02	0.88
Plant part (P)	2	85.45	<b>&lt;0.0001</b>	4	10.61	<b>0.0001</b>
G×P	2	1.71	0.23	4	1.10	0.39

Table 4.2 Plant height (cm) measured at the V7, R5 and R7 growth stages and ANOVA in Experiment 1.

Treatment		V7		R5		R7	
		mean(±SE) (cm)					
Bt	-CRW	191.45((±4.85)		238.28(±5.47)		227.33(±7.37)	
	+CRW	-		246.70(±6.38)		233.05(±5.23)	
NonBt	-CRW	207.65((±6.16)		246.83(±6.60)		240.03(±2.92)	
	+CRW	-		263.37(±5.92)		245.75(±4.84)	
Source of Variance	df	F-value	p-value	F-value	p-value	F-value	p-value
Genotype (G)	1	6.56	<b>0.03</b>	4.26	<b>0.05</b>	7.52	<b>0.01</b>
CRW (C)	1	1.33	0.28	4.17	<b>0.05</b>	1.52	0.23
G × C	1	4.24	0.07	0.44	0.51	0.00	1.00

Plant part, CRW presence and the interaction between plant part and CRW presence significantly affected acid detergent lignin concentration (ADL %) measured at the R5 stage ( $p < 0.05$ ), while genotype had no effect on ADL (Table 4.4). At R5, roots had the highest lignin concentration, followed by cobs, stalks, leaves, and husks (Figure 4.3 A). The average ADL concentration of (+)CRW plants was 4.35%, while that of (–)CRW plants was 4.72%. The ADL concentration of (–)CRW stalks and cobs was significantly higher than that of (+)CRW stalks and cobs ( $p < 0.05$ ), respectively. For shoot parts (stalks, leaves, cobs and husks), pest presence increased average lignin concentration. In contrast, the average ADL concentration of (+)CRW roots was higher than that of (–)CRW roots.

After plants were labeled at R2 and R4 stages, the  $^{13}\text{C}$  content of whole plants or roots measured at the R5 stage did not differ significantly by genotype or CRW presence (Table 4.5 and 4.6). The percentages of labeled  $^{13}\text{C}$  allocated to different plant parts are shown in Figure 4.4. The labeled  $^{13}\text{C}$  allocated to plant parts differed significantly ( $p = 0.0001$ ), in the following order: husks < leaves < cobs < stalks < roots on average.

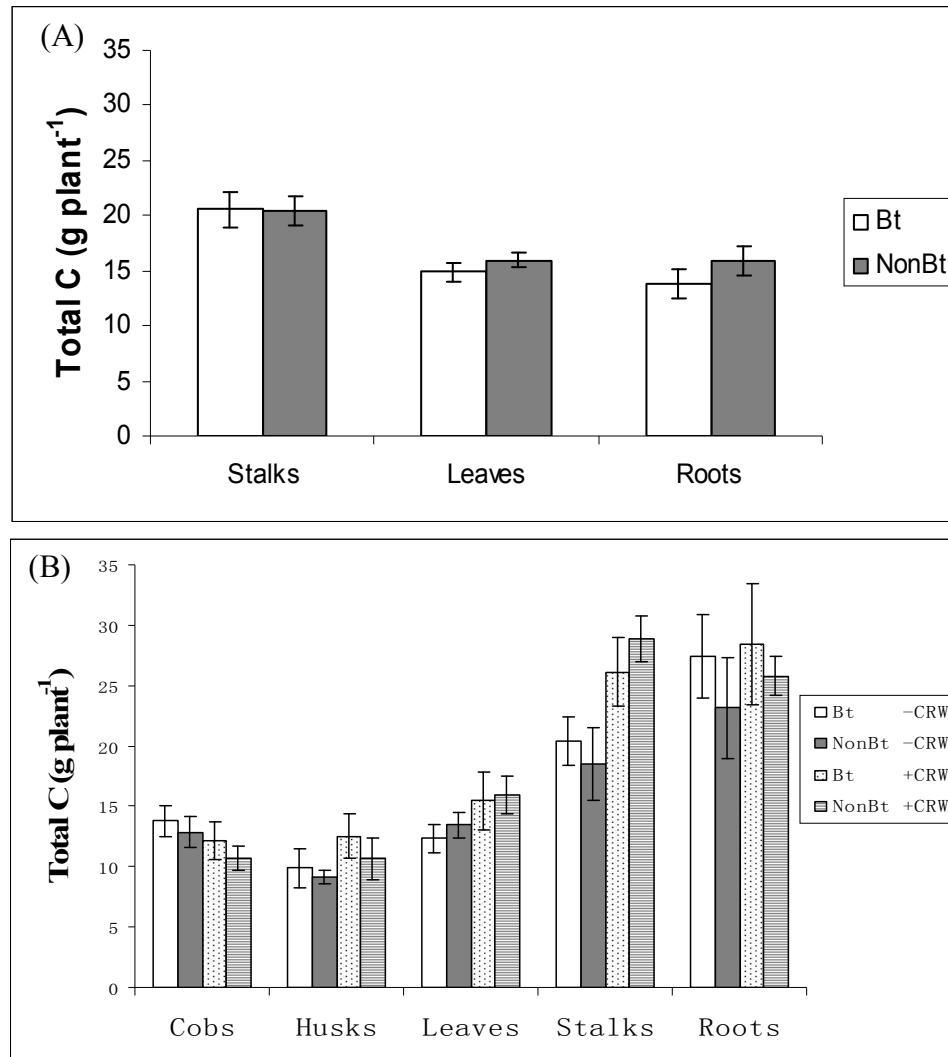


Figure 4.2 Total C allocated to different plant parts in Bt and NonBt corn measured at the (A) V7 and (B) R5 stages of development with (+) or without (-) CRW presence in Experiment 1 (error bars: SE).

Table 4.3 ANOVA for total C content measured at the V7 and R5 stages in Experiment 1.

Source of Variance	V7			R5		
	df	F-value	p-value	df	F-value	p-value
<b>All plant part</b>						
Genotypes (G)	1	0.98	0.33	1	0.90	0.35
CRW (C)	1	0.01	0.94	1	6.64	<b>0.01</b>
Plant part (P)	2	13.86	<b>&lt;0.0001</b>	4	39.45	<b>&lt;0.0001</b>
G×C	1	1.80	0.19	1	0.16	0.69
G×P	2	0.43	0.65	4	0.57	0.69
C×P	2	0.14	0.87	4	2.56	<b>0.05</b>
G×C×P	2	0.36	0.70	4	0.29	0.88

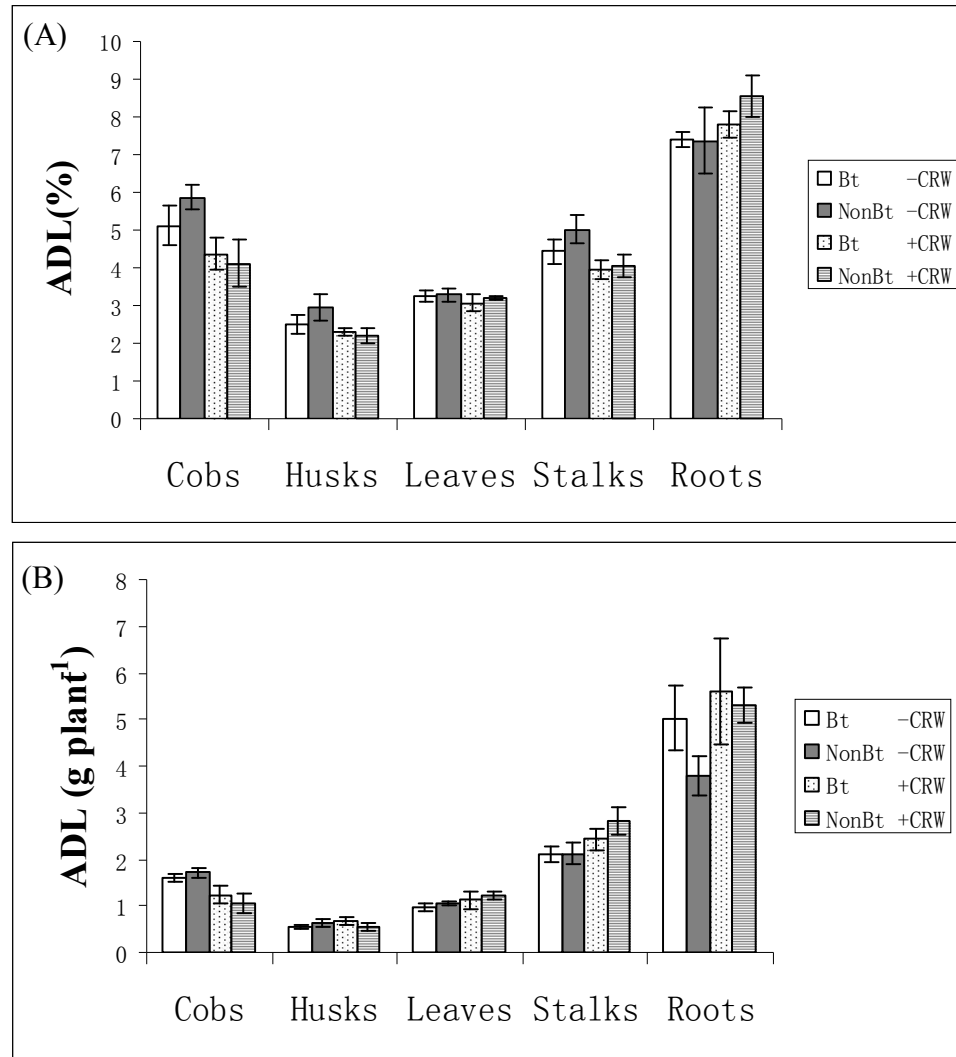


Figure 4.3 Acid Detergent Lignin (ADL) (A) concentration and (B) content measured at the R5 stage in Bt and NonBt corn with (+) or without (-) CRW pest presence in Experiment 1 (error bars: SE).

Table 4.4 ANOVA for Acid Detergent Lignin (ADL) concentration and content measured at the R5 stage in Experiment 1.

Source of Variance	ADL Concentration			ADL Content		
	df	F-value	p-value	df	F-value	p-value
Genotypes (G)	1	2.12	0.15	1	0.51	0.48
CRW (C)	1	4.59	<b>0.04</b>	1	2.61	0.11
Plant part (P)	4	116.64	<b>&lt;0.0001</b>	4	105.29	<b>&lt;0.0001</b>
G×C	1	0.51	0.48	1	0.33	0.57
G×P	4	0.09	0.98	4	1.26	0.30
C×P	4	4.09	<b>0.01</b>	4	2.99	<b>0.03</b>
G×C×P	4	0.82	0.52	4	0.55	0.70

Table 4.5  $^{13}\text{C}$  content of whole plants measured at the R5 stage and ANOVA in Experiment 2.

Treatment		$^{13}\text{C}$ content [g $^{13}\text{C}$ plant $^{-1}$ ( $\pm$ SE)]
Bt	-CRW	0.05 ( $\pm$ 0.03)
	+CRW	0.04 ( $\pm$ 0.01)
NonBt	-CRW	0.03 ( $\pm$ 0.01)
	+CRW	0.03 ( $\pm$ 0.01)

Source of Variance	df	F-value	p-value
Genotype (G)	1	0.88	0.38
CRW (C)	1	0.17	0.69
G $\times$ C	1	0.11	0.75

Table 4.6  $^{13}\text{C}$  content in roots measured at the R5 stage and ANOVA in Experiment 2.

Treatment		$^{13}\text{C}$ content in roots [g $^{13}\text{C}$ plant $^{-1}$ ( $\pm$ SE)]
Bt	-CRW	0.029 ( $\pm$ 0.024)
	+CRW	0.011 ( $\pm$ 0.003)
NonBt	-CRW	0.012 ( $\pm$ 0.007)
	+CRW	0.004 ( $\pm$ 0.001)

Source of Variance	df	F-value	p-value
Genotype (G)	1	0.92	0.37
CRW (C)	1	0.91	0.37
G $\times$ C	1	0.17	0.69

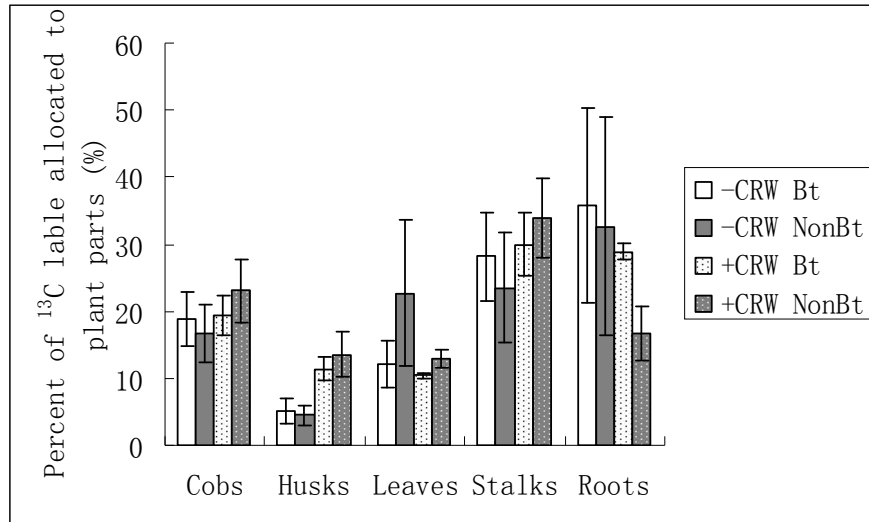


Figure 4.4 Allocation of labeled  $^{13}\text{C}$  to Bt and NonBt corn plant parts with or without CRW at the R5 stage in Experiment 2 (error bars: SE).

Table 4.7 ANOVA for  $^{13}\text{C}$  allocation to Bt and NonBt corn plant parts with or without CRW at the R5 stage in Experiment 2.

Source of Variance	ADL Concentration		
All plant parts	df	F-value	p-value
Genotypes (G)	1	0.00	1.00
CRW (C)	1	0.00	1.00
Plant part (P)	4	7.91	<b>0.0001</b>
G×C	1	0.00	1.00
G×P	4	0.61	0.65
C×P	4	1.43	0.24
G×C×P	4	0.39	0.81
<b>Husks</b>			
Genotype (G)	1	0.60	0.46
CRW (C)	1	<b>25.62</b>	<b>0.0015</b>
G × C	1	0.86	0.39

For individual plant parts, significantly more labeled  $^{13}\text{C}$  was allocated to (+)CRW husks than (-)CRW husks ( $p = 0.0015$ ) (Table 4.7). There was no significant effect of genotype or CRW on labeled  $^{13}\text{C}$  allocation of any other plant part.



### **4.3.3 Experiment 3: Comparison of Above and Below-Ground Biomass in Corn from Three Maturity Groups**

This experiment was conducted to assess whether biomass differences between Bt and NonBt were comparable to the variation that might be observed typically for varieties in different MGs. Aboveground biomass (stalks+leaves) of the 96 d Bt and NonBt corn in MG-A was significantly greater than that of corn in the other two maturity groups at the V5 stage ( $p = 0.06$ ) by orthogonal contrasts (Table 4.8). As shown in Figure 4.4 A, the only genotype effect on aboveground biomass was observed in the 101 d MG-B, where Bt leaves and stalks weighed more ( $p = 0.09$ ) than NonBt leaves and stalks. However, the ratio between above and belowground biomass did not differ between or within the three MGs (Figure 4.5 B).

## **4.4 Discussion**

### **4.4.3 Plant Growth**

The only effect of genotype (Bt or NonBt) on the variables measured in these experiments was on plant height in Experiment 2 ( $p < 0.05$ ). NonBt corn was significantly taller than Bt corn at the V7, R5 and R7 stages, suggesting that the observed height differences may be a result of inherent differences in the parental lines used in this study. The process by which transgenic plants are developed makes it nearly impossible to obtain a NonBt counterpart of a Bt line that differs only in this marker (Thomas Nickson, Monsanto Co, pers. comm.). This lack of ability to directly compare corn varieties with and without the Cry3Bb1 gene inserted was the primary reason that we evaluated Bt and NonBt varieties in different maturity groups. This allowed us to determine whether the differences observed between Bt and NonBt corn were comparable to the variation observed between different maturity groups

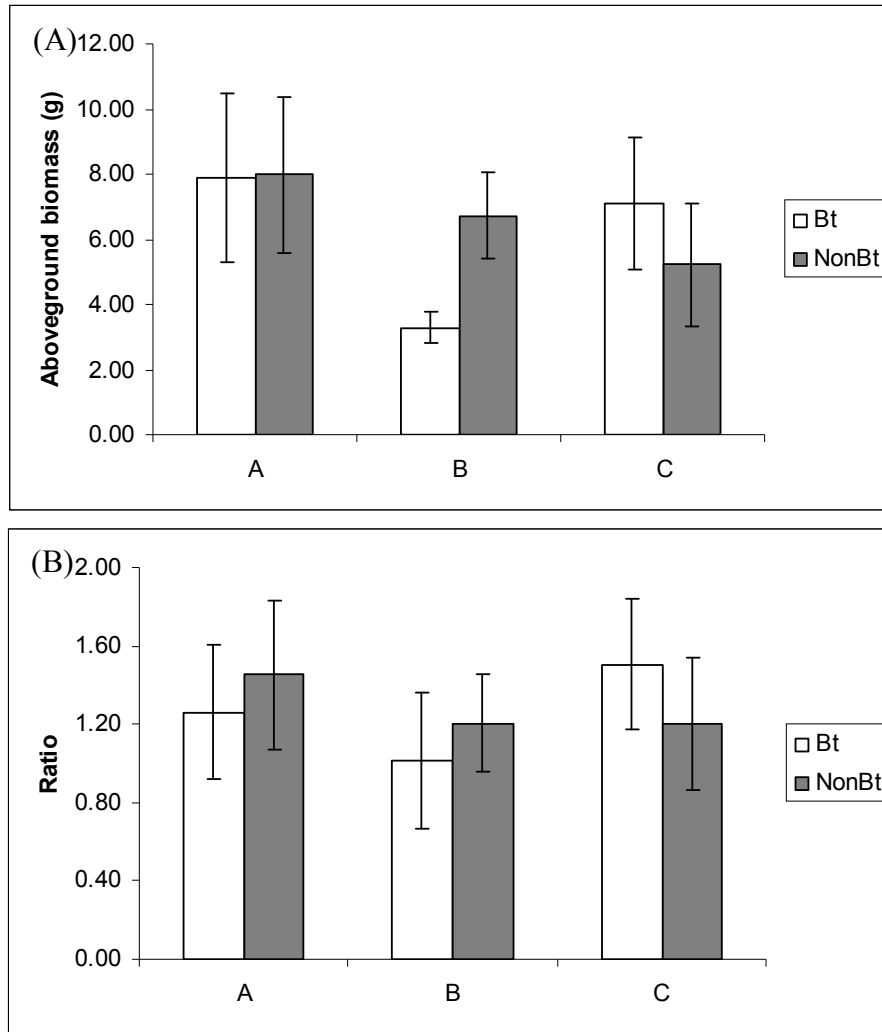


Figure 4.5 Comparison of (A) aboveground biomass and (B) the ratio between above and belowground biomass for Bt and NonBt corn in maturity groups A = 96 d; B = 101 d; and C = 111 d (error bars: SE) in Experiment 3.

Table 4.8 Orthogonal contrasts of aboveground biomass and the ratio between above and belowground biomass at the V5 stage for Bt and NonBt corn in maturity groups A= 96 d; B = 101 d; and C = 111 d in Experiment 3.

Maturity Group Contrasts	Aboveground Biomass				Ratio			
	Estimate	SE	t-value	p-value	Estimate	SE	t-value	p-value
A vs B and C	-9.40	4.60	-2.04	<b>0.06</b>	0.50	1.01	-0.50	0.63
B vs C	2.31	2.66	0.87	0.40	0.48	0.58	0.83	0.42
Bt vs NonBt in A	0.10	1.88	0.06	0.96	0.19	0.41	0.46	0.65
Bt vs NonBt in B	3.43	1.88	1.82	<b>0.08</b>	0.19	0.41	0.47	0.65
Bt vs NonBt in C	-1.89	1.88	-1.00	0.33	-0.31	0.41	-0.74	0.47

(Experiment 3). In Experiment 3, a difference was observed in aboveground biomass between the 96 d MG-A and the other two MGs ( $p = 0.06$ ), while there was no significant difference between the Bt and NonBt genotypes within MG-A. Thus, the difference in aboveground biomass between transgenic and non-transgenic corn in MG-A was less than the differences in biomass observed between the different MGs.

Among the three maturity groups, Bt corn had a different above ground biomass ( $p = 0.09$ ) than that of its NonBt counterpart only in MG-B. Differences between genotypes were also observed by Griffiths *et al.* (2007), who found that Cry1Ab Bt maize had a significantly greater shoot C:N ratio than its NonBt counterpart for two of eight paired lines.

As variations in aboveground biomass and any other variables measured in this study may also exist between traditional, non-transgenic hybrids, which have not been tested as intensively as TCs, it may not be necessary to test TCs further if the aboveground biomass or other variables of Bt corn are within the same range as that of traditional hybrids.

#### **4.4.2 Carbon Allocation**

The percentage of labeled  $^{13}\text{C}$  allocated to different plant parts represents the short-term C allocation during the labelling periods. In Experiment 2, there were no significant effects of genotype (Bt or NonBt) on total C content or short-term  $^{13}\text{C}$  allocation, indicating that C allocation was not affected by the presence of the Cry3Bb1 gene. The lack of any differences suggests that the presence of the Cry3Bb gene may not significantly affect C budgets in agricultural systems.

The  $^{13}\text{C}$  pulse-labeling in Experiment 1 indicated that corn plants respired or sloughed off about 53.1% of the  $^{13}\text{C}$  label about 48 d after labeling. The  $^{13}\text{C}$  content in roots did not change significantly, indicating that C was being allocated to roots by

remobilization or that respiration and sloughing losses of C were minor. The release of exudates from roots may have contributed to the  $^{13}\text{C}$  dilution observed; however, it did not change the  $^{13}\text{C}$  content in roots. In Experiment 2,  $^{13}\text{C}$  content was measured at the R5 stage, 26 days after the first labeling at the R2 stage. Based on the results in Experiment 1, it is possible that  $^{13}\text{C}$  content in plant parts other than roots was respired or sloughed off before the measurements taken at the R5 stage. The  $^{13}\text{C}$  content in roots might be a more reliable indicator as it did not change significantly with time. In Experiment 2, genotype did not have a significant effect on  $^{13}\text{C}$  content in roots.

In the second harvest of Experiment 2, 48 days after the target hatching date of CRW, a significantly higher height and total C content of plants inoculated with CRW was found ( $p < 0.05$ ). Moreover, significantly more labeled  $^{13}\text{C}$  was allocated to (+)CRW husks than (-)CRW husks ( $p = 0.0015$ ). This suggested that (+)CRW plants suffered only minor grazing, which may have caused induced systemic resistance (ISR), a mechanism for plants to protect themselves, which can stimulate growth and change C allocation within plants (Feldman, 1984; Wild, 1988; Waisel *et al.*, 1991). I postulate that limited damage occurred from the initial grazing of the larvae on Bt plants, resulting in ISR for these treatments as well.

#### **4.4.3 Lignin Concentration and Content**

There were no significant effects of genotype (Bt or NonBt) on ADL concentration or content, indicating that presence of the Cry3Bb gene may not significantly affect C turnover in agricultural systems. There is no obvious mechanism I am aware of that would lead to a change in plant lignin content in Cry3Bb corn, as the gene transformed into Bt corn plants is not part of the lignin biosynthetic pathway (Jung and Sheaffer, 2004). However, it is theoretically possible to have pleiotropic effects resulting from the transformation event as are expected in any hybridization event (Snow *et al.*, 2005).

Our results are in accord with a laboratory study conducted by Hopkins and Gregorich (2003) and several field experiments using Cry1Ab Bt corn (Jung and Sheaffer, 2004; Zwahlen *et al.*, 2007). In a field study, Lehman *et al.* (2008) buried litterbags containing residues (stalks+leaves) from Cry3Bb, Cry1Ab, stacked Cry1Ab/Cry3Bb, and control NonBt corn in a wheat field for 22 months and also did not find any significant effect of genotype on lignin concentration or decomposition rate. However, higher lignin concentration and/or a slower decomposition rate of Cry1Ab Bt corn as compared with non-transgenic lines have also been reported (Saxena and Stotzky, 2001; Castaldini *et al.*, 2005; Flores *et al.*, 2005; Fang *et al.*, 2007). These conflicting lignin concentration results might be due to inherent difference between the different hybrids used by different researchers. Moreover, the comparison of lignin concentrations between Bt and NonBt genotypes could also be affected by different plant growth conditions.

Although the presence of the Bt gene did not alter corn lignin content, the lignin content in (–)CRW NonBt roots was significantly lower than that of (+)CRW NonBt roots at the R5 stage, while there was no significant difference for Bt roots with or without CRW added. It has been reported that ISR in plants can increase the formation of protective biopolymers, including lignin. The tomato *PAL* (*Arabidopsis PAL*) gene, *Arabidopsis CHS* gene and *Arabidopsis TAT* gene were found to regulate lignin synthesis that strengthened the cell wall in plant defense or as a wound response (Kombrink and Somssich, 1997; Reymond and Farmer, 1998; Reymond *et al.*, 2000). Since the presence of CRW only affected corn roots, the increased lignin content of NonBt roots without protection from CRW feeding might be explained by ISR, but it will require further work to verify this.

## 4.5 Conclusions

There were no significant effects of genotype (Bt or NonBt) on total C content,  $^{13}\text{C}$  allocation, or lignin concentration or content, indicating that the Bt transgenic event in MON863 corn is unlikely to have a significant effect on soil C budgets in agricultural lands. However, corn rootworm presence in Experiment 2 resulted in higher total C content and plant height in infested plants, including both Bt and NonBt hybrids. Induced systemic resistance is suggested as an explanation for these observations.

Lignin content of NonBt roots increased without protection from CRW feeding compared to NonBt roots without CRW added. However, there was no significant difference for Bt roots with or without CRW added. This indicates that the presence of CRW may affect root lignin content. The protection from CRW feeding provided by the expression of Bt gene, not the transformation, were responsible for the difference observed between Bt and NonBt corn. This different reactions to CRW by Bt and NonBt plants may influence carbon budgets in agricultural systems when CRW is present.

NonBt corn was significantly taller than Bt corn at the V7, R5 and R7 stages of development, but this is likely the result of normal varietal variation as suggested also by the results of the experiment in which variation between 3 MGs and between Bt and NonBt corn within each maturity group was compared. Aboveground biomass was higher in the 96 d MG compared to the 101 and 111 d MGs ( $p = 0.06$ ). No significant difference was observed between the Bt and NonBt hybrids within the 96 d MG, indicating that the difference between Bt and NonBt within that MG was less than differences between MGs. Aboveground biomass differed between Bt and NonBt only within the 101 d MG.

These results indicate that variables measured for Bt corn in this study were within

the same range as those for NonBt corn. Thus, it may not be necessary to exhaustively test these variables for TCs further. Moreover, considering the environmental benefits that TCs can achieve, the decision to test should also depend on the agriculture practices that the TCs are substituted for.

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**CHAPTER 5**  
**ATTITUDES TOWARD TRANSGENIC CROPS: A CASE STUDY OF**  
**CONSUMERS AND FARMERS IN BEIJING AND JIANGSU PROVINCES,**  
**CHINA**

**Abstract**

A perception study was conducted in China to explore the attitudes of Chinese consumers towards transgenic crop (TC) development and use. A questionnaire was developed and 1772 respondents were surveyed at shopping areas in Beijing (large city), Guanyun (small village) in Jiangsu Province, universities and graduate schools. Different focus groups, including 37 farmers, participated in extended interviews as well. Results showed that the AWARENESS of consumers about TCs was low in Guanyun, but relatively high in Beijing and extremely high for university students. The percentage of opponents to genetically modified organism (GMO) RESEARCH or GMO APPLICATIONS was quite low, but a large proportion of respondents held neutral positions or were not sure. Opponents were more insistent about their position than supporters were, but those who were holding a neutral position were more likely to change their position to be more negative than positive. Respondent's attitudes were shifted by communicating either the benefits or potential risks of GMOs. In interviews, farmers said that IR rice may not be attractive to them as they were concerned about the cost and effectiveness of seeds. Farmers surveyed in this study were more interested in TCs that would use less fertilizer than in IR rice. Consumer choice behavior after receiving symmetrical information should help the industry develop more effective marketing strategies. My results indicated that the commercial release of IR rice may encounter consumer resistance. Predictions for future scenarios suggest that consumer AWARENESS is likely to increase with time but that ACCEPTANCE might

decrease as suggested by the predictive models. Government regulatory bodies need to be aware of consumer attitudes and take them into account when developing policies for the commercial release of IR rice. If the benefits are perceived to be limited and IR rice seeds are not attractive to farmers, the government might chose to focus on transgenic rice that can use less fertilizer, which was the preferred option for meeting farmers' needs in this study.

## **5.1 Introduction**

Transgenic crops (TCs) were first approved for commercial production in 1996. Since then, the global area planted with TCs has increased steadily at a double-digit rate for several consecutive years, reaching 114.3 million ha in 2007 (James, 2007). TCs can provide some environmental, economic, health and social benefits (James, 2007). Insect resistant (IR) TCs, for example, can reduce pesticide use on crops, which has serious detrimental effects on human health and the environment (Ando and Khanna, 2000). Brookes and Barfoot (2006) estimated that the cumulative reduction in pesticide use by growing IR crops was 289,000 Mt of active ingredients (a.i.) globally from 1996 to 2006. Based on the Environmental Impact Quotient (EIQ) developed by Kovach *et al.* (1992 and updated annually), the associated environmental impact of pesticide use on those areas planted with IR crops decreased 15.5% during that period. However, concerns continue to be raised by the public about the potential environmental and health effects of TCs.

In China, Bt cotton, virus-resistant plants (tomato, sweet pepper, chili pepper and papaya), delayed ripening tomato, and color-altered petunia have been approved for commercial planting by the Office of Genetic Engineering Safety Administration (OGESA) in the Ministry of Agriculture (MOA) (OGESA, 2007). Among these TCs, only Bt cotton has achieved commercial success. In 2007, 7.1 million small and

resource-poor farmers planted 3.8 million ha of Bt cotton, accounting for 69% of all cotton planted in China (James, 2007). Although transgenic cotton is not used for food, Chinese consumers do obtain food in local markets that contains transgenic ingredients derived from TCs, such as imported soybean oil. China is the world's largest importer of soybeans, most of which are genetically modified (herbicide tolerant) (Ho and Vermeer, 2004).

Rice is among the more important food crops in the world and is a staple food in China. In 2006, 29.3 million ha of rice were planted in China, accounting for 20% of the global rice area (James, 2007) and 18.7% of the total arable land in China (STATS, 2007). As revealed by the Deputy Director of the Science, Technology and Education Division in the MOA, the bio-safety evaluations of IR rice "are at the last stage" (China Daily, 2008). The OGESA will consider approving the commercial release of transgenic rice after this process is finished. The possible approval of IR rice will have a more profound effect on respondents' lives than any other TC. Unsurprisingly, this has stimulated a major debate that is being played out in the Chinese media (Keeley, 2006).

The ATTITUDE of consumers will be among the more important factors determining the ACCEPTANCE and economic success of commercially released transgenic rice in the market. Farmers' attitudes and their willingness to adopt IR rice are also critical for success as farmers are more involved in this issue than any other group. Attitudes of farmers and consumers need to be considered in the governmental decision-making process.

Previous studies in the U.S. have reported conflicting results concerning consumer attitudes about GM food (GMA, 2000; Lusk, 2003; IFIC, 2006; Pew Initiative, 2006). This could be explained, in part, by how different questions were worded. Specific wording is known to have a strong influence on the answers to different questions

posed (Hoban, 2004). Rowe (2004) pointed out that “the public acceptance of GM foods is much more complex than simply communicating their benefits”. Various surveys in China showed that Chinese consumers have a low AWARENESS of, but relatively strong support for biotechnology (Gale *et al.*, 2002; Bai, 2003; Li *et al.*, 2003; AFIC, 2003), or a low level of opposition (Zhong *et al.*, 2002). However, Greenpeace, a famous opponent of GM foods (Xinhua News Agency, online news, 2005) reported greater opposition and less support for transgenic crops in Guangzhou. Moreover, Ho and Vermeer (2004) predicted future scenarios of resistance against transgenic food in China.

In this study, I investigated the ATTITUDE of Chinese consumers towards transgenic rice by using questionnaire surveys and interviewing different groups of people, including farmers. I also determined the important factors affecting respondents’ attitudes towards TCs. During this study, I did not advocate for or against agricultural biotechnology or transgenic crops, but held a neutral position, and provided symmetrical information to respondents about both the benefits and risks of TCs. The effect of information biased more toward either the benefits or the potential risks of IR rice on respondents’ attitudes was also examined.

## **5.2 Methods**

A questionnaire was developed to assess respondents’ AWARENESS, ACCEPTANCE, and WILLINGNESS TO PAY (WTP) for or WILLINGNESS TO ACCEPT (WTA) transgenic foods, especially IR rice (see Appendix B). Symmetrical information, including both benefits and risks, was provided to respondents in a rotating order.

In Beijing, China, 702 valid questionnaires were collected randomly from different shopping areas (shopping Beijing = SB). Among these questionnaires, 228 were completed outside the Bajia Market located in the suburbs, which represented a



lower-income socio-economic class (LOW); 204 questionnaires were completed outside Chaoshifa Supermarket, which represented a middle-income class (MIDDLE); and 177 questionnaires were completed outside shopping malls, indicating a relatively higher-income class (HIGH). Another 93 questionnaires were completed in a CLUB located in a shopping mall (CLUB), which represented the middle or higher-income classes. In Jiangsu Province, 543 consumers were surveyed in the shopping areas in Guanyun (shopping Guanyun = SG), a small rural town. Except in the Beijing CLUB, all questionnaires were collected immediately after they were completed. In the CLUB, the questionnaires were left in its office and collected after one week.

In order to investigate the next generation of consumers with higher EDUCATION levels, 527 valid questionnaires were collected randomly from four universities and one graduate school in Beijing (University = UN). The 100 students surveyed at China Agricultural University, were presumed to have strong backgrounds in agriculture; 97 students surveyed were from Renmin University, and were expected to have strong backgrounds in the social sciences; 100 Tsinghua and 99 Peking University students were surveyed who had a variety of different academic backgrounds. Moreover, 120 Master of Science degree students were surveyed from the Graduate School of the Chinese Academy of Sciences (GSCAS). They had various majors in science or engineering. All of these questionnaires were collected immediately after they were finished.

Multinomial, binary, and ordinal logistic regression models were applied to the data using SPSS v16 (SPSS Inc, Chicago, IL) in different analyses. The Chi-Square Test was used to test the relationships between the dependent variable and a combination of independent variables to gather 'model fitting' information. The Likelihood-Ratio test was used to measure the statistical significance of each

independent variable used in the model. The Wald Test was applied to evaluate the statistical significance of each independent variable in each of the embedded binary logistic comparisons. The function for the logistic regression model used is:

$$\Pr(y_i = j) = \frac{\exp(X_i\beta_j)}{1 + \sum_j^J \exp(X_i\beta_j)}$$

Where, for the  $i$ th individual,  $y_i$  was the observed outcome and  $X_i$  was a vector of explanatory variables. The predictive parameter,  $\beta_j$ , was estimated by the maximum likelihood method. All statistical analyses, including descriptive analyses, excluded missing data (questions that respondents chose not to answer). Statistical significance was considered to be at  $\alpha=0.05$ .

In addition, 37 farmers in Beijing and 30 farmers in Guanyun, Jiangsu Province, were interviewed to assess their AWARENESS and understanding of TCs. Four government officers, five NGO members, three policy scientists and 18 scientists in biology or environmental science were interviewed as well.

## 5.3 Results and Discussion

### 5.3.1 Questionnaire

#### 5.3.1.1 Demographic Information

A total of 1772 valid questionnaires were obtained in three LOCATIONS (SG, SB and UN) that had quite different demographic characteristics. In my study, 53% of respondents were MALE and 47% were FEMALE. For the whole Chinese population, the National Bureau of Statistics of China (NBSC) reported that males accounted for 51.5% of the total population in 2006 (STATS, 2007). In my study, 51.9% of respondents were 18-26 years old. This differed from the national AGE structure reported in 2006 (Figure 5.1), which was distributed more evenly. Not surprising, 96.9% of those from

universities and GSCAS were 18-26 years old. In SB and SG, such percentages were 47.1% and 9.1%, respectively. Most respondents (75.9%) in Guanyun were 25-44 years old.

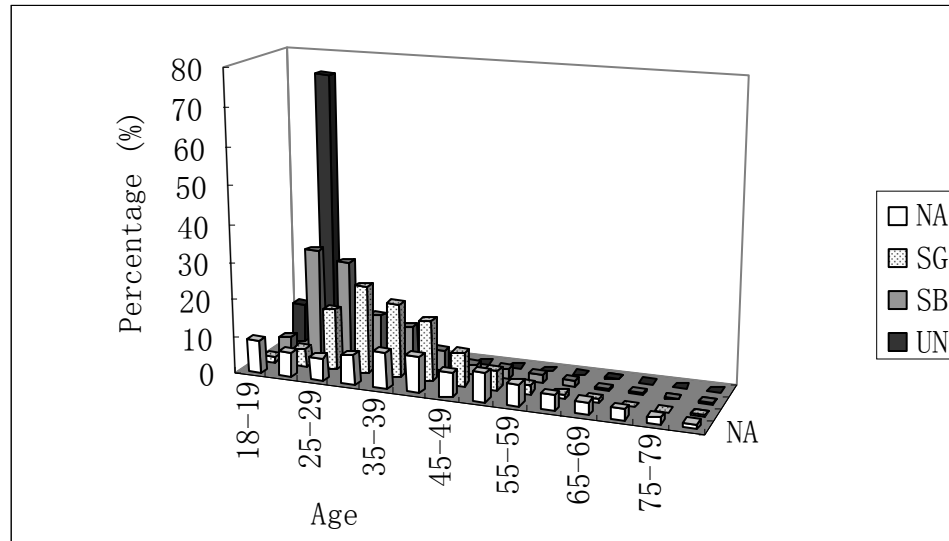


Figure 5.1 AGE distribution of respondents in SG, SB and UN compared to the national (NA) in 2006.

In my study, 46.7% of the respondents had Bachelors' degrees. In SB, only 13.8% had an EDUCATION level below high school, but 48.6% had their Bachelors' degrees. EDUCATION levels differed in the shopping areas that represented different socio-economic classes. The number of respondents with an EDUCATION level below high school was highest in the LOW socio-economic neighborhood and decreased in the order: LOW>MIDDLE>CLUB>HIGH. (Figure 5.2 A).

Consumers in Beijing had higher EDUCATION levels than in Guanyun, where 40.9% of the respondents had EDUCATION levels below high school. The number of respondents with higher levels of EDUCATION were more numerous in my study compared with the national average (NA), for which 80.9% had an EDUCATION level below high school in 2006 (Figure 5.2 B). Such percentages in Beijing and Jiangsu Province reported by the NBSC were 47.5% and 77.6%, respectively, in 2006 (STATS,

2007).

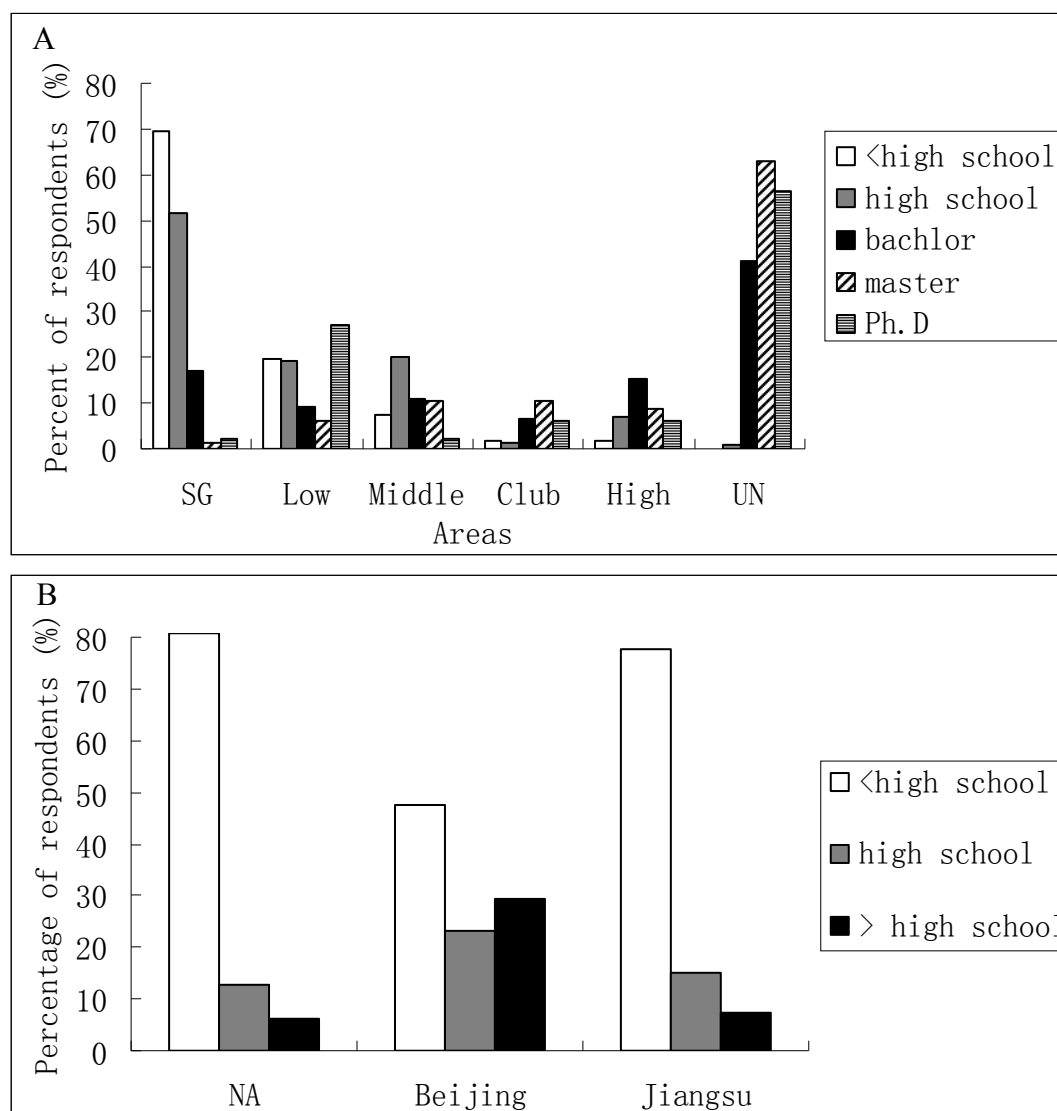


Figure 5.2 EDUCATION level distribution in: (A) the different LOCATIONS studied; and (B) the National Average (NA) in 2006 compared to Beijing and Jiangsu respondents.

A total of 11.5% respondents claimed that they had a BIOLOGY BACKGROUND. Over one-third (35.5%) of them could answer the question “where is the Bt gene in Bt crops from” correctly, accounting for 45.3% of 159 correct answers. A total of 11.7% of the respondents had environmental NGO EXPERIENCE within five years of the time of survey.

The national average monthly INCOME in China in 2006 was 1750 RMB (US \$256), while average INCOME in Beijing and Jiangsu were higher, 3343 and 1982 RMB (US \$489 and \$290), respectively (STATS, 2007). In my study, 28.1% of the respondents refused to provide their INCOME information. Excluding these missing data, 52.9% earned less than 4499 RMB (US \$658) monthly. The INCOME level of respondents in Beijing was obviously higher than in Guanyun (Figure 5.3). However, respondents could mix-up the concepts of personal income and household income easily and tend not to answer the INCOME question honestly because of security considerations, especially in a public place. Because of concerns about validity and the large proportion of missing data, INCOME information was not included in the multinomial or ordinal logistic regression models as a predictor variable.

#### **5.3.1.2 AWARENESS**

A total of 21.7% of the respondents said that they had heard nothing about genetically modified organisms (GMOs) or did not know even after a reminder. A few (8.3%) realized that they had heard of GMOs before this survey, after hearing a short description of what they are. A total of 70.0% of the respondents thought they had heard of GMOs, more or less, before this survey without any reminder. These percentages in SG, SB and UN were 42.0% 74.4% and 94.4%, respectively (Figure 5.3A). Overall, the AWARENESS level of consumers surveyed was high in Beijing, but still low in Guanyun. For students surveyed from universities/graduate school, the AWARENESS level was extremely high.

In an ordinal logistic regression model, the variables that significantly ( $p < 0.05$ ) affected AWARENESS included AGE, EDUCATION, environmental NGO EXPERIENCE, BIOLOGY BACKGROUND and LOCATION in the Wald Test (Table 5.1). There was a significant inverse relationship between AGE and AWARENESS, meaning that the

Table 5.1 Parameter estimates for AWARENESS using an ordinal logistic regression.

<i>X</i>			
Explanatory Variables	Estimate $\beta$	Std. Error	Sig.
AGE	-0.012	0.005	0.030
EDUCATION	0.758	0.063	0.000
NGO EXPERIENCE: -/+	-0.493	0.161	0.002
GENDER: MALE/FEMALE	0.047	0.097	0.627
BIOLOGY BACKGROUND BACKGROUND: -/+	-1.039	0.171	0.000
LOCATION: SB VS. UN	-0.291	0.135	0.031
SG VS. UN	-1.155	0.176	0.000

probability of having a higher AWARENESS level decreased with increasing AGE (Figure 5.3B). The high mean AWARENESS value for 70-74 or 75-80 year old respondents could be attributed to the small sample size of the two groups, 4 and 2 people, respectively. In contrast, this probability increased significantly with increasing EDUCATION (Figure 5.3C). With a BIOLOGY BACKGROUND or NGO EXPERIENCE, the probability of having a greater AWARENESS level was significantly higher (Figure 5.3D, E). The probability of having a higher AWARENESS level was in the order of UN > SB > SG (Figure 5.3A).

AGE was inversely related to the AWARENESS level. One important explanation of why younger people were more aware is that GMOs have been introduced into junior high school biology textbooks since 2003. It was not surprising that the probability of having a higher AWARENESS level was significantly greater for respondents with a BIOLOGY BACKGROUND, environmental NGO EXPERIENCE or high EDUCATION levels because those groups were more likely to be exposed to information about GMOs. LOCATION was also a significant factor affecting AWARENESS. INCOME and EDUCATION levels were different between Beijing and Guanyun (Figure 5.2). The AGE structures

were also different between consumers and university students (Figure 5.1). The factors of cultures and/or accessibility to information may explain these effects. A low AWARENESS of GMOs by Chinese consumers has been reported in some studies (Gale *et al.*, 2002; Bai, 2003; Li *et al.*, 2003; AFIC, 2003). However, the results of my study showed that the AWARENESS levels differed by LOCATIONS and a low AWARENESS was only observed in the rural village, Guanyun. In Beijing, 44.9% of consumers surveyed had heard something or a great deal about GMOs, a similar percentage to that reported in various surveys conducted from 1992 to 2003 for American consumers (Hoban, 1996, 2004; IFIC, 2001). Comparing results within the same LOCATION, Beijing, Li *et al.* (2003) found that 90% of 599 respondents had no or little knowledge of biotechnology, while the relevant percentage in my study was much lower, 52.5%. Li *et al.* (2003) also conducted a survey through personal interviews in shopping areas. This increase in AWARENESS from their study in 2002 and completed in 2008 and mine conducted in 2008 may indicate that the AWARENESS level is a dynamic variable, changing with time.

Only 9.0% of all respondents answered correctly that the Bt gene in Bt crops was from bacteria, indicating that respondents' actual knowledge about GMOs was still limited. The percentage of correct answers increased with the level of what respondents thought they knew about GMOs (Figure 5.4). The Pew Initiative on Food and Biotechnology (2006) also found that in-depth knowledge about GM foods was clearly very limited among American consumers. Just 26% thought that they had eaten GM foods in 2006, but the fact is that most Americans, if not all, have eaten GM foods. Up to 70% of processed products in American grocery stores are produced "using some form of biotechnology or genetic modification" (Pew Initiative, 2006).

Figure 5.3 AWARENESS: How much have you heard about GMOs? AWARENESS levels (A) in different LOCATIONS; (B) mean values with AGE; (C) with EDUCATION; (D) with or without environmental NGO EXPERIENCE; (E) with or without BIOLOGY BACKGROUND.



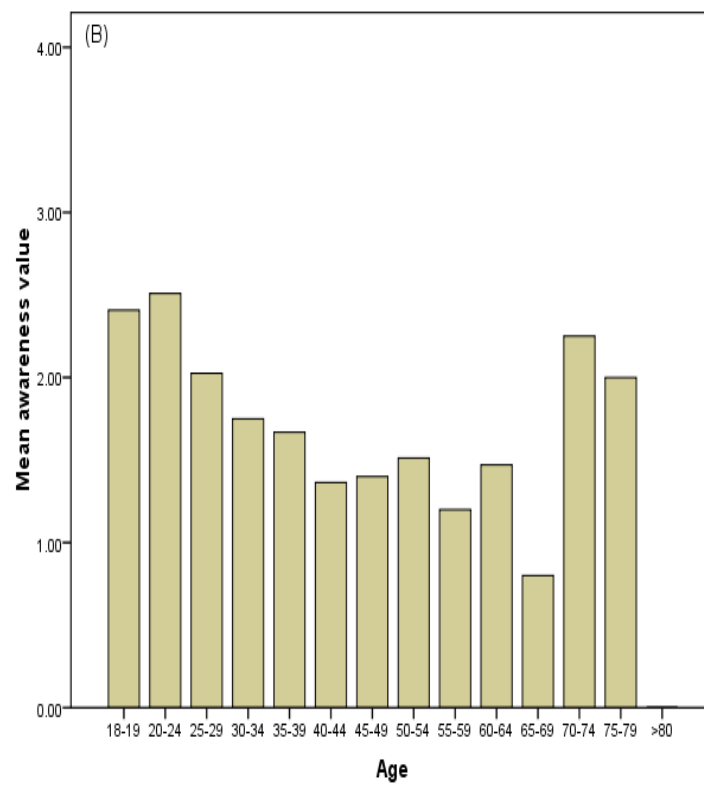
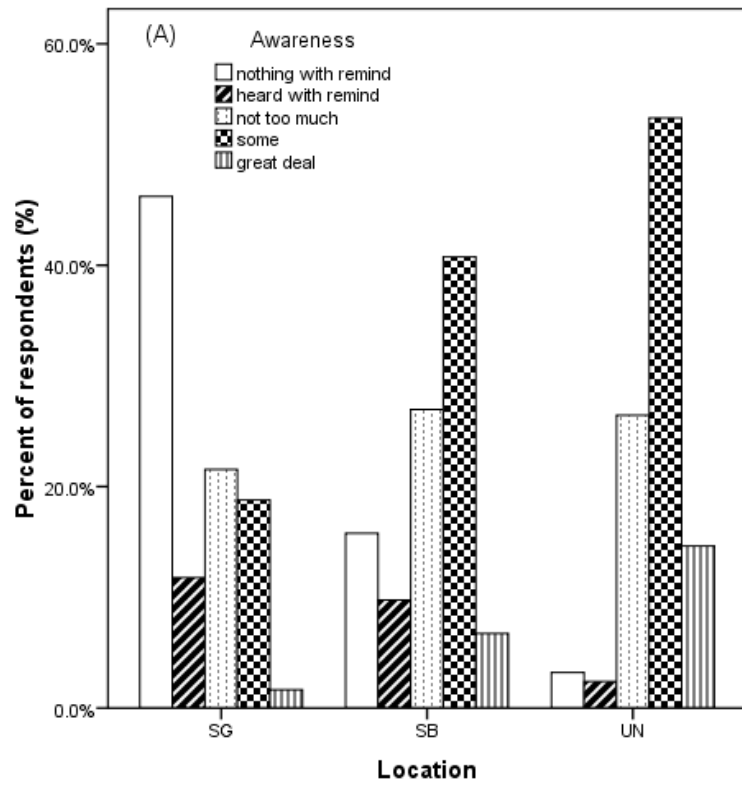


Figure 5.3

Figure 5.3 (continued)

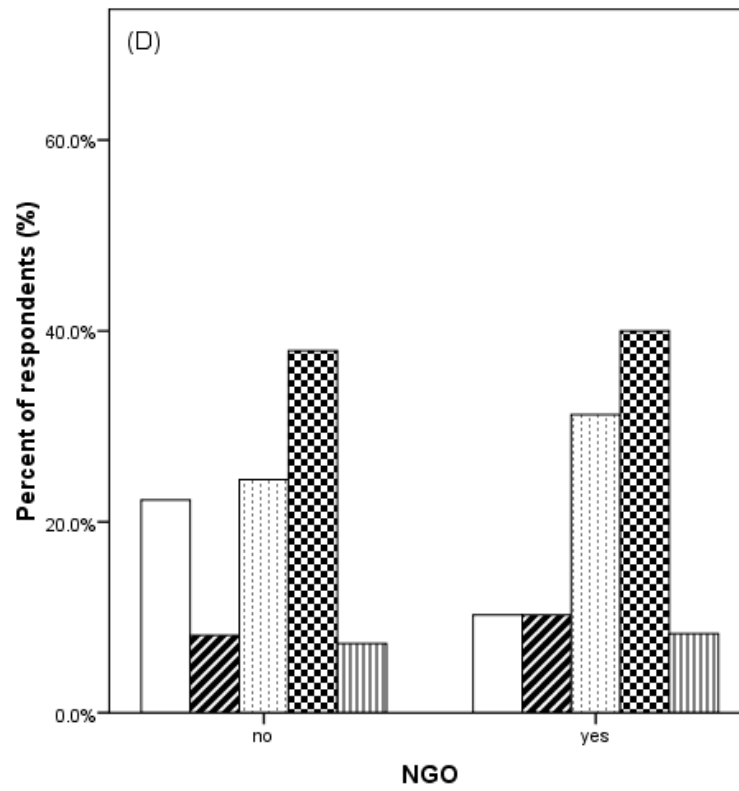
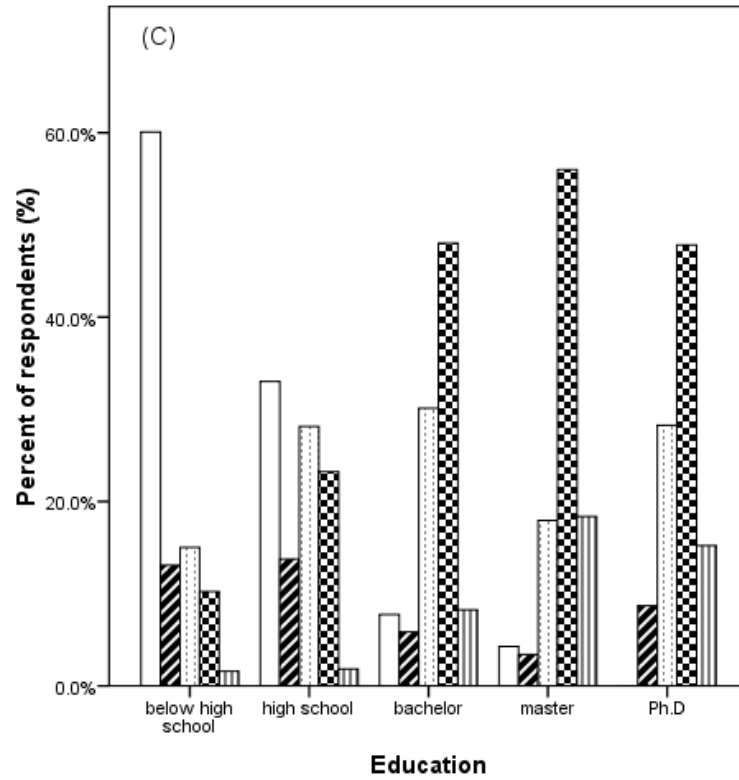
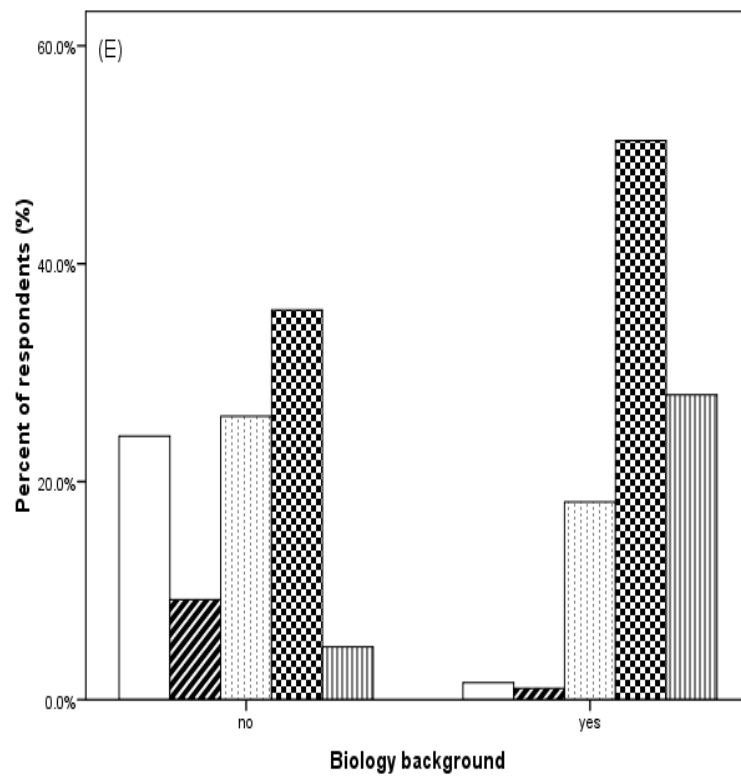


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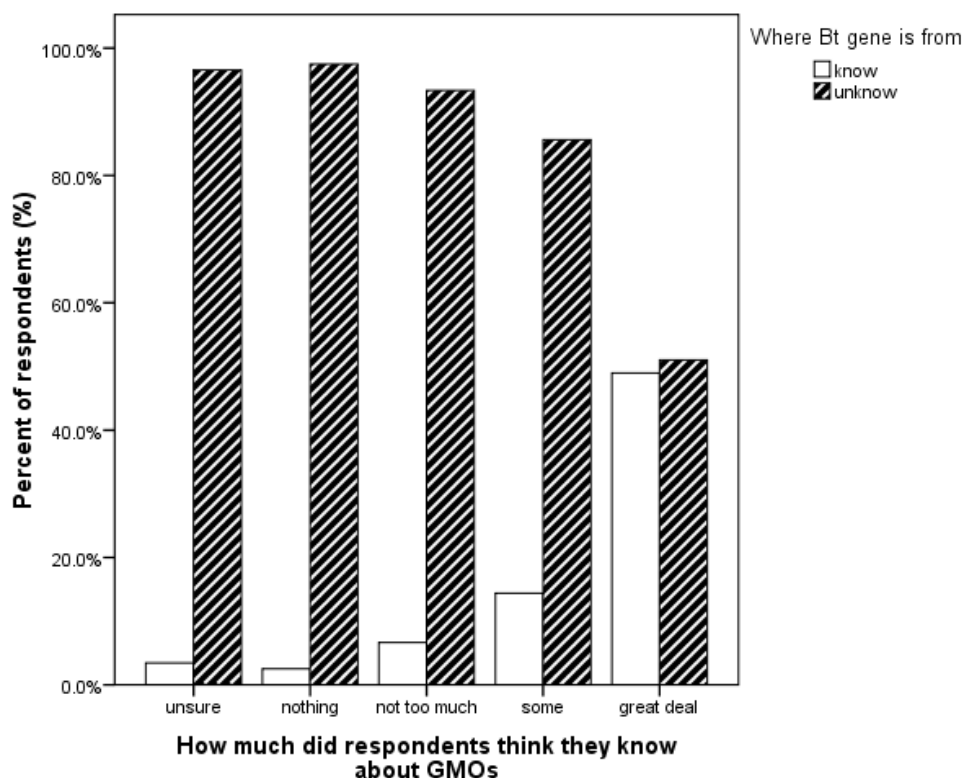


Figure 5.4 “How much do you think you know about GMOs?” compared with the knowledge level of the source of the Bt gene.

### 5.3.1.3 ACCEPTANCE

Respondents had a more positive attitude towards GMO RESEARCH than GMO APPLICATIONS. Almost two-third (62.8%) of respondents supported GMO RESEARCH while only 4.3% were against it, to some degree; 18.0% held a neutral position and 15.0% were unsure. For GMO APPLICATIONS, fewer supporters (40.5%) and more opponents (10.7%), neutral respondents (29.3%) and unsure respondents (19.5%) were found. In the UN, SB and SG test groups, 75.2, 67.2 and 45.1% supported GMO RESEARCH, respectively, while only 4.7, 4.7 and 3.4% were opposed. For GMO APPLICATIONS, the percentages of supporters decreased to 39.0, 45.8 and 33.8% in the UN, SB and SG test groups, respectively, while the percentages of opponents were 13.8, 14.2 and 1.4%, respectively (Table 5.2). These different reactions to GMO RESEARCH vs

GMO APPLICATIONS confirmed a point made by Hoban (2004) that respondent's responses are very sensitive to the wording of the questions asked.

In some other studies, interviewers observed a very high ACCEPTANCE of Chinese consumers towards applications of "biotechnology". Gale *et al.* (2002) reported the percentage of supporters was 79% in 1999. Li *et al.* (2003) also found a prevailing positive opinion (63%). The differences could be explained by the different wording used. The applications of "biotechnology" used in surveys conducted by Gale *et al.* (2002) and Li *et al.* (2003), was much more acceptable to consumers than the term "GMO" used in my study (Hoban, 2004).

The low percentage of opponents to GMO RESEARCH or GMO APPLICATIONS was consistent with the findings of Zhong *et al.* (2002) and Lin *et al.* (2003) for Chinese consumers. Compared to American consumers in 2006 (Pew Initiative, 2006), opposition to GMOs was much lower and support for GMOs was relatively higher in my study. In the Pew Initiative (2006) study, researchers addressed the question of introducing GM food into the U.S. food supply, reflecting the commercialization (GMO APPLICATION) of GMO technology. The Eurobarometer survey in 2005 showed that GM food was predominantly perceived negatively by Europeans and a majority thought that "GM food should not be encouraged" (Gaskell *et al.*, 2006). In contrast to the U.S. and Europe, the low opposition to GMO APPLICATION observed in China could be attributed to a recent period of rapid industrialization. McCluskey *et al.* (2003) claimed that "a highly desired and rapid" economic development has made the Chinese more forward-looking and new technologies "are often considered much needed improvements" in China. During the process of answering questions, Chinese respondents might not perceive any direct effect of GMOs on their own lives. Even if GM products were available, they might assume that they would have the choice not to select them. This may explain, in part, why respondents did not want to become

obstacles to “advanced-technology”.

The trust level for the current food system could be another possible explanation. However, the trustworthiness rating of government regulators was slightly higher in the U.S. (Pew Initiative, 2006) than it was in China, according to my results. A total of 14% of American consumers trusted government regulators “a great deal” (Pew Initiative, 2006), while 12% of Chinese respondents trusted the government “a great deal” in my study. For the Food and Drug Administration (FDA) in the U.S., this rating was much higher at 29%. Since respondents in my study did not trust the Chinese government any more than U.S. respondents did theirs (Pew Initiative, 2006), the level of trust people have in their government is unlikely to explain the low opposition observed in China. In concert with this, the Eurobarometer survey in 2005 pointed out that their results did not support the claim that “there is a crisis of trust in actors involved in biotechnology in Europe” (Gaskell *et al.*, 2006).

For those supporting GMO RESEARCH, 61.3% also supported GMO APPLICATIONS, but 23.7% switched to a neutral position and 8.8% were not sure. For those neutral about GMO RESEARCH, 74.5% kept the same position when asked about GMO APPLICATIONS, but 17.3% became against. The vast majority of unsure respondents (96.8%) and opponents (86.7%) of GMO RESEARCH did not change their positions for GMO APPLICATIONS (Figure 5.5). This may indicate that opponents were more insistent upon their position than supporters were; whereas neutral and unsure respondents were more likely to be opposed than supportive towards GMO APPLICATIONS. The large proportion of neutral/unsure attitudes observed in this study suggests the possibility of consumer resistance to GMO APPLICATIONS in the future.

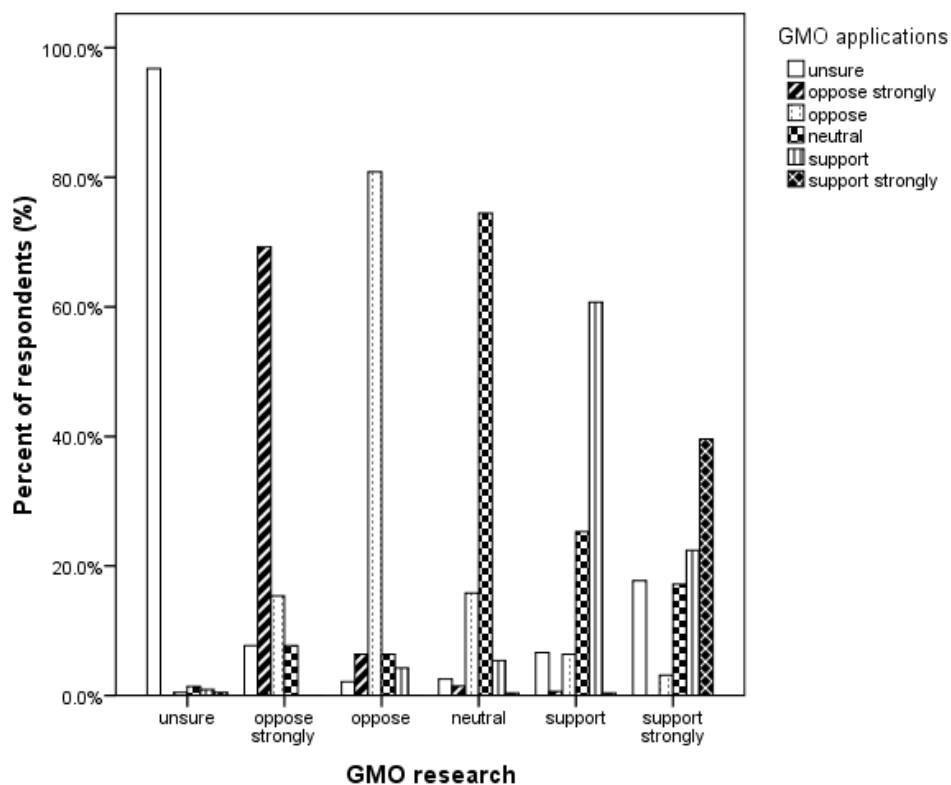


Figure 5.5 ACCEPTANCE: “Generally, do you favor or oppose the development of GMOs by scientists or their application in agriculture or industry?” ACCEPTANCE of GMO RESEARCH vs. GMO APPLICATIONS.

Table 5.2 ATTITUDE structure (% of respondents) for GMO RESEARCH, GMO APPLICATION and commercial release of IR rice.

ATTITUDE	SG			SB			UN		
	RE	AP	IR rice	RE	AP	IR rice	RE	AP	IR rice
Unsure	36.2	49.0	32.7	8.1	11.1	11.3	2.0	6.0	6.5
Oppose	3.4	1.4	4.7	4.7	14.2	16.9	4.7	13.8	20.7
Neutral	15.3	15.7	21.7	20.0	28.9	30.7	18.1	41.3	38.6
Support	45.1	33.8	40.9	67.2	45.8	41.0	75.2	39.0	34.2

Note: RE-RESEARCH; AP-APPLICATION



A binary logistic regression model was used to analyze the binary dependent variables, that is, whether respondents were SURE or UNSURE about their position. The variables EDUCATION, AWARENESS, NGO EXPERIENCE and LOCATION were significant for ATTITUDE toward GMO RESEARCH (Table 5.3), while AWARENESS, NGO EXPERIENCE, GENDER and LOCATION were significant variables related to ATTITUDE toward GMO APPLICATIONS in the Wald Test at  $p < 0.05$  (Table 5.4). The probability of being UNSURE about either GMO RESEARCH or GMO APPLICATIONS decreased significantly as AWARENESS increased (Figure 5.6 A, B). The probability of being UNSURE for respondents without environmental NGO EXPERIENCE was significantly higher (Figure 5.6 C, D). The probability of being UNSURE about GMO RESEARCH or GMO APPLICATIONS was in the order of  $SG > SB > UN$  survey groups (Table 5.2). For GMO APPLICATIONS, this probability in SG was significantly higher than in UN, but there was no significant difference between the SB and UN test groups. The probability of being UNSURE about GMO RESEARCH decreased significantly with increasing EDUCATION level (Figure 5.6 E). FEMALES, compared to MALES, had a significantly higher probability of being UNSURE about GMO APPLICATIONS (Figure 5.6 F).

For the subgroup of respondents who were SURE of their position, an ordinal logistic regression model was used. For GMO RESEARCH, BIOLOGY BACKGROUND and LOCATION were significant variables ( $p < 0.05$ ) in the Wald Test (Table 5.3). With BIOLOGY BACKGROUND, the probability of having a higher supportive level was significantly higher (Figure 5.7A). Compared to the UN samples, this probability for SB samples was significantly lower. There was no significant difference between the UN and SG samples (Figure 5.7B). For GMO APPLICATIONS, AGE, EDUCATION, AWARENESS, GENDER, BIOLOGY BACKGROUND and LOCATION were significant variables ( $p < 0.05$ ) in the Wald Test (Table 5.4).

Table 5.3 Parameter estimates for ACCEPTANCE of GMO RESEARCH. The reference category is SURE.

<i>X</i> Explanatory Variables	Binary logistic regression model <sup>a</sup> : sure or not sure			Ordinal logistic regression for respondents who were sure their position		
	Estimate $\beta$	Std. Error	Sig.	Estimate $\beta$	Std. Error	Sig.
AGE	-0.003	0.009	0.713	-0.006	0.006	0.362
EDUCATION	-0.311	0.116	0.007	-0.014	0.073	0.851
AWARENESS	-0.619	0.084	0.000	-0.062	0.055	0.264
NGO EXPERIENCE: -/+	0.905	0.379	0.017	-0.050	0.184	0.785
GENDER: MALE/FEMALE	-0.181	0.178	0.308	0.210	0.113	0.064
BIOLOGY BACKGROUND: -/+	0.082	0.637	0.898	-0.544	0.178	0.002
LOCATION: SB VS. UN	0.914	0.448	0.041	-0.358	0.149	0.016
SG VS. UN	2.228	0.463	0.000	-0.100	0.205	0.626

Table 5.4 Parameter estimates for ACCEPTANCE of GMO APPLICATIONS. The reference category is SURE.

<i>X</i> Explanatory Variables	Binary logistic regression model <sup>a</sup> : sure or not sure			Ordinal logistic regression for respondents who were sure their position		
	Estimate $\beta$	Std. Error	Sig.	Estimate $\beta$	Std. Error	Sig.
AGE	-0.013	0.009	0.127	-0.013	0.006	0.047
EDUCATION	-0.075	0.105	0.477	-0.348	0.075	0.000
AWARENESS	-0.508	0.074	0.000	-0.136	0.058	0.019
NGO EXPERIENCE: -/+	0.602	0.305	0.049	-0.336	0.190	0.076
GENDER: MALE/FEMALE	-0.319	0.163	0.050	0.606	0.116	0.000
BIOLOGY BACKGROUND: -/+	-0.039	0.357	0.914	-0.345	0.169	0.042
LOCATION: SB VS. UN	0.428	0.275	0.120	0.007	0.145	0.961
SG VS. UN	2.275	0.311	0.000	0.521	0.225	0.020

Figure 5.6 ACCEPTANCE: Generally, do you favor or oppose the development of GMOs by scientists or their APPLICATION in agriculture or industry? Acceptance for GMOs: (A) GMO RESEARCH with AWARENESS; (B) GMO APPLICATIONS with AWARENESS; (C) GMO RESEARCH with or without environmental NGO EXPERIENCE; (D) GMO APPLICATIONS with or without environmental NGO EXPERIENCE; (E) GMO RESEARCH with EDUCATION; (F) GMO APPLICATIONS vs. GENDER.

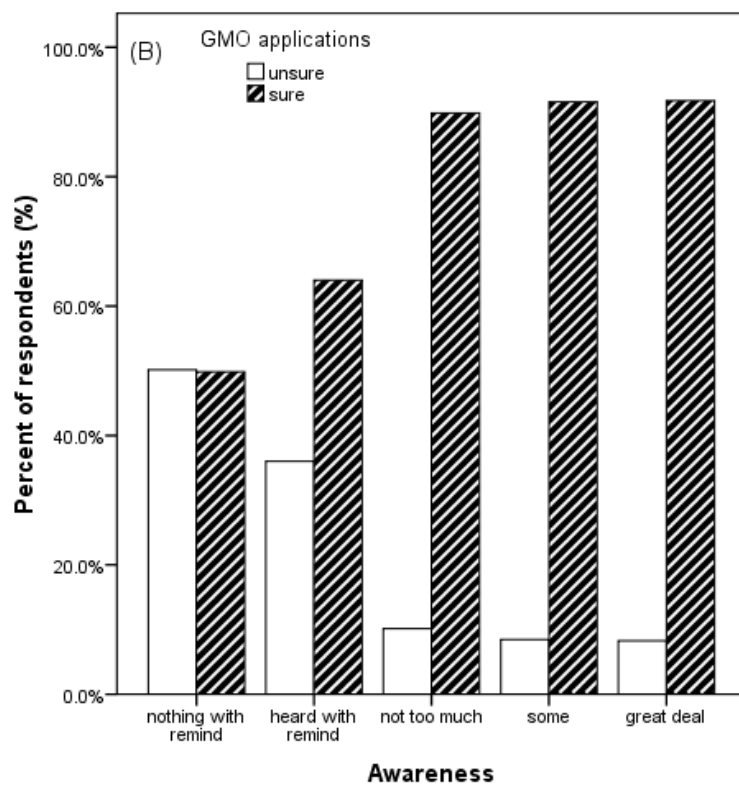
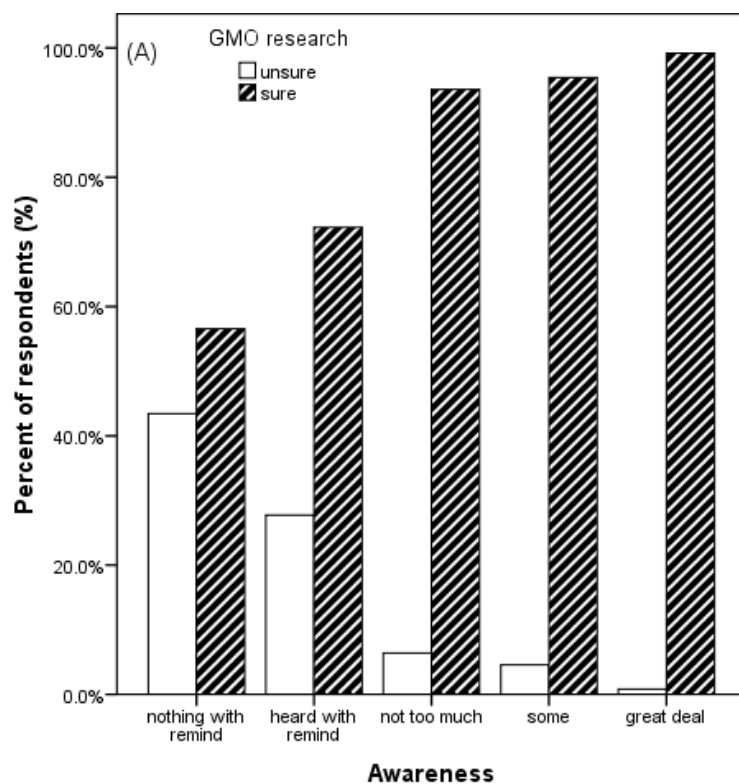


Figure 5.6

Figure 5.6 (continued)

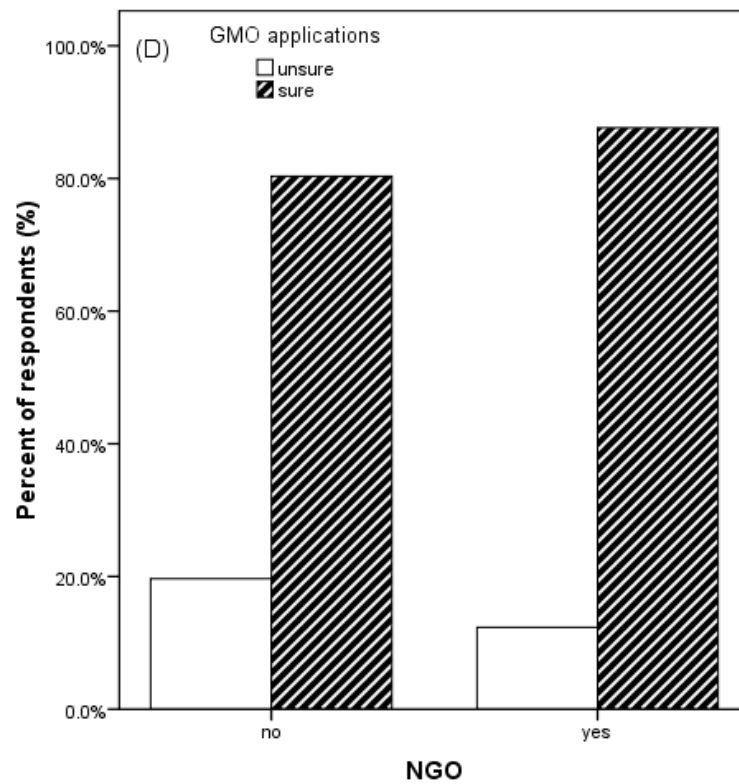
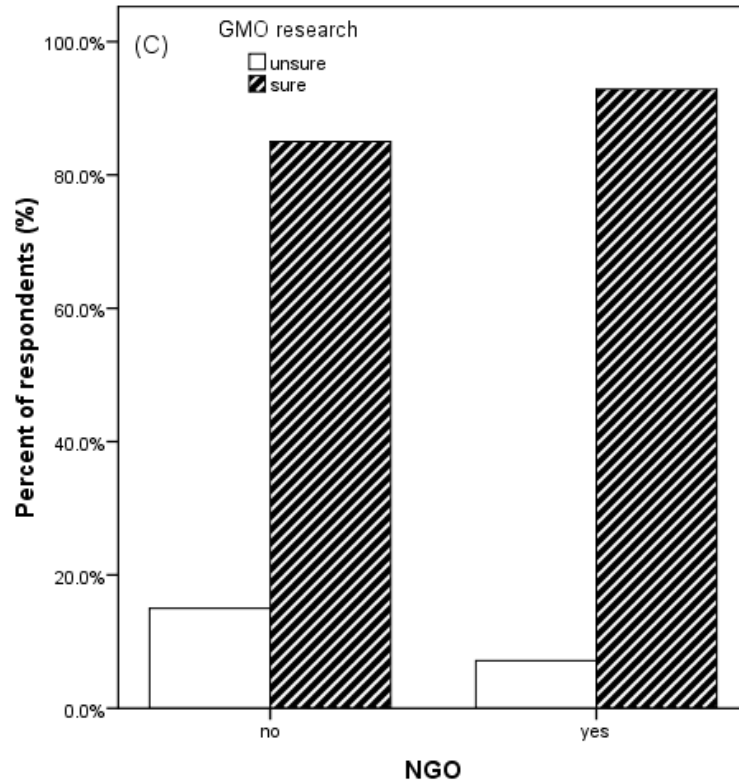
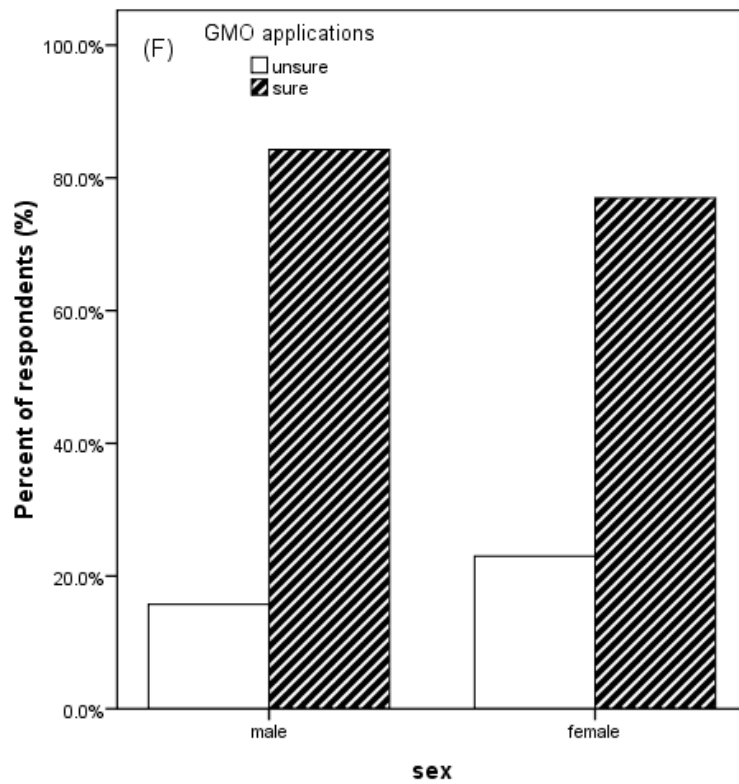
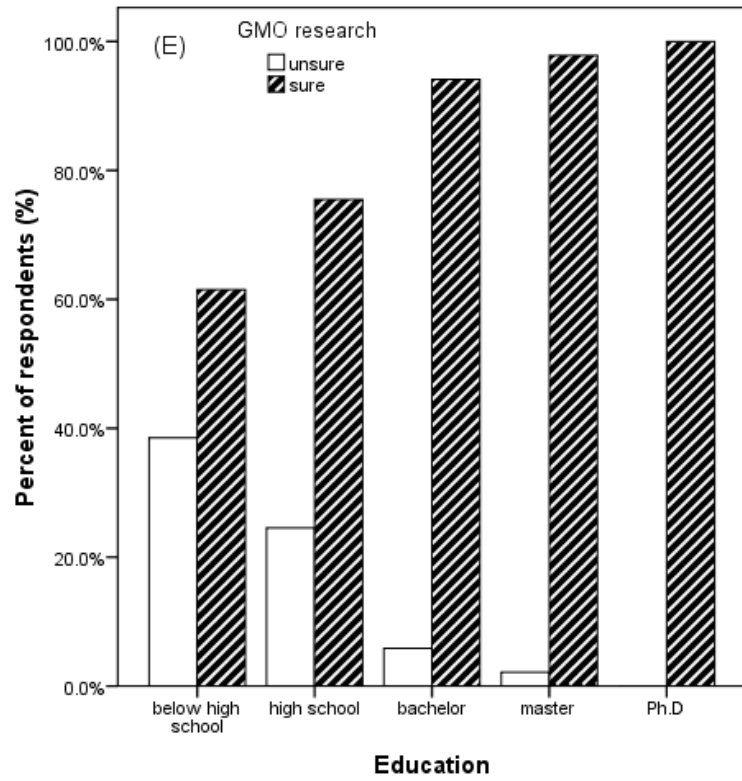


Figure 5.6 (continued)



Respondents' ACCEPTANCE of GMO APPLICATIONS was inversely related to EDUCATION and AWARENESS levels. This meant that the probability of having a higher supportive level decreased with increasing EDUCATION and AWARENESS levels (Figure 5.7 C, D). MALES had a significantly higher probability of being more supportive (Figure 5.7 E). Compared to the UN samples, SG samples had a significantly higher probability of having a higher supportive level while SB samples did not (Figure 5.7 F). Respondents' responses didn't differ significantly by the single factors of AGE or BIOLOGY BACKGROUND.

MALES had a significantly higher probability of being certain about their position and to be more supportive. This was consistent with the surveys by the Pew Initiative (2006) in the U.S. and by Zhong *et al.* (2002) in Nanjing, China. In my study, the probability of being more supportive decreased with the increasing AGE. This was consistent with Li *et al.* (2003) and Lusk (2003). These results reflected that FEMALES and older people tended to be more cautious. However, Zhong *et al.* (2002) found that older respondents tended to accept GM food more than younger respondents. The reason for this conflicting result is still unknown. With a higher EDUCATION or AWARENESS level, the probability of being more supportive of GMO APPLICATIONS was significantly lower. This group of respondents may have recognized that new technologies could pose risks to human health and/or the environment based on past lessons. So, their ATTITUDES were relatively conservative, especially when they had limited information or knowledge. However, respondents with a BIOLOGY BACKGROUND had a significantly higher probability of being more supportive, consistent with my results from interviews with scientists. These predicted that respondents' ACCEPTANCE could be increased by EDUCATION with in-depth knowledge of biology.

Figure 5.7 ACCEPTANCE of GMO RESEARCH and GMO APPLICATIONS: subgroup of respondents who were SURE of their position. ACCEPTANCE for (A) GMO RESEARCH with or without BIOLOGY BACKGROUND; (B) GMO RESEARCH in different LOCATIONS; (C) GMO APPLICATIONS with EDUCATION; (E) GMO APPLICATIONS with AWARENESS; (E) GMO APPLICATIONS vs. GENDER; (F) GMO APPLICATIONS in different LOCATIONS.



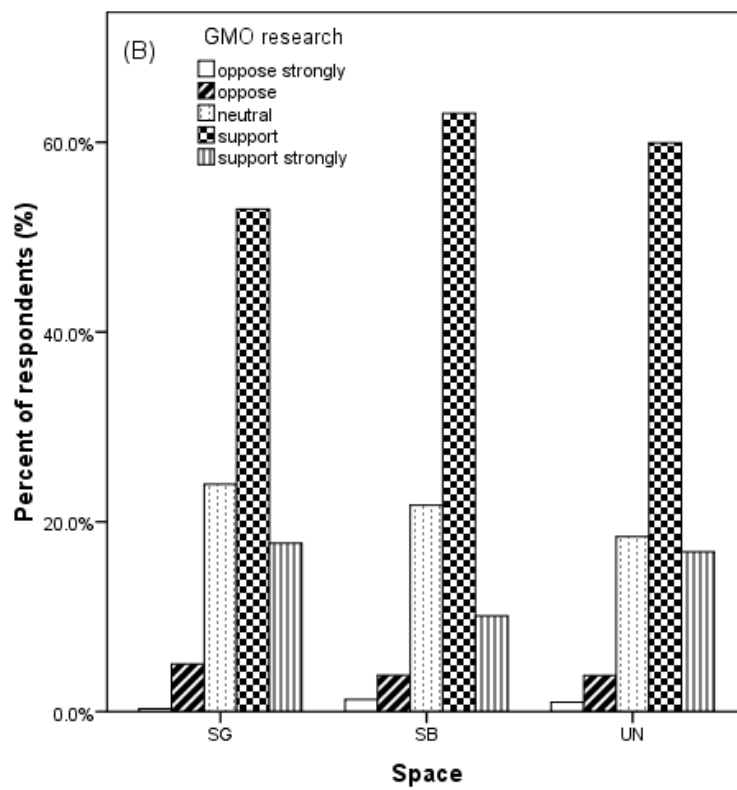
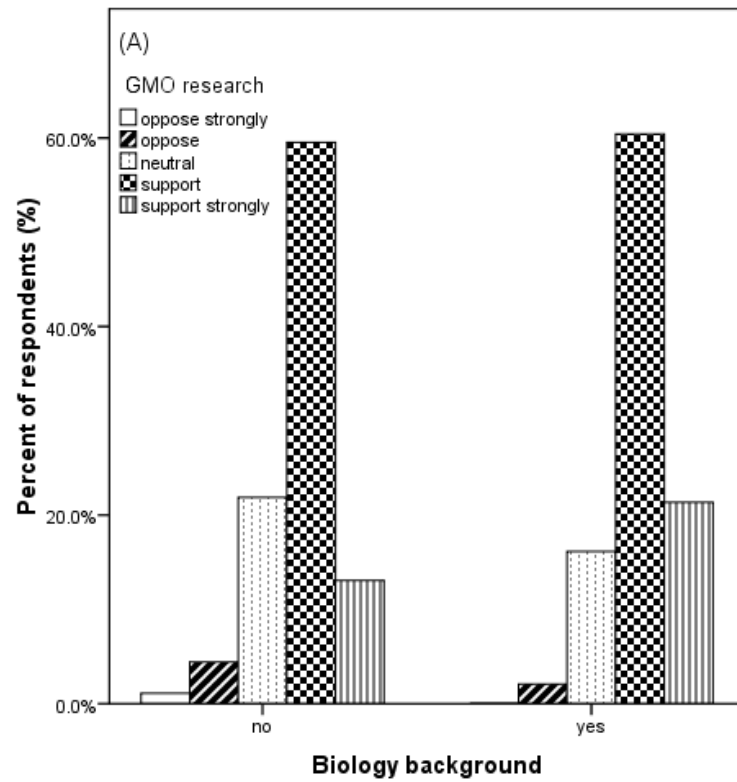


Figure 5.7

Figure 5.7 (continued)

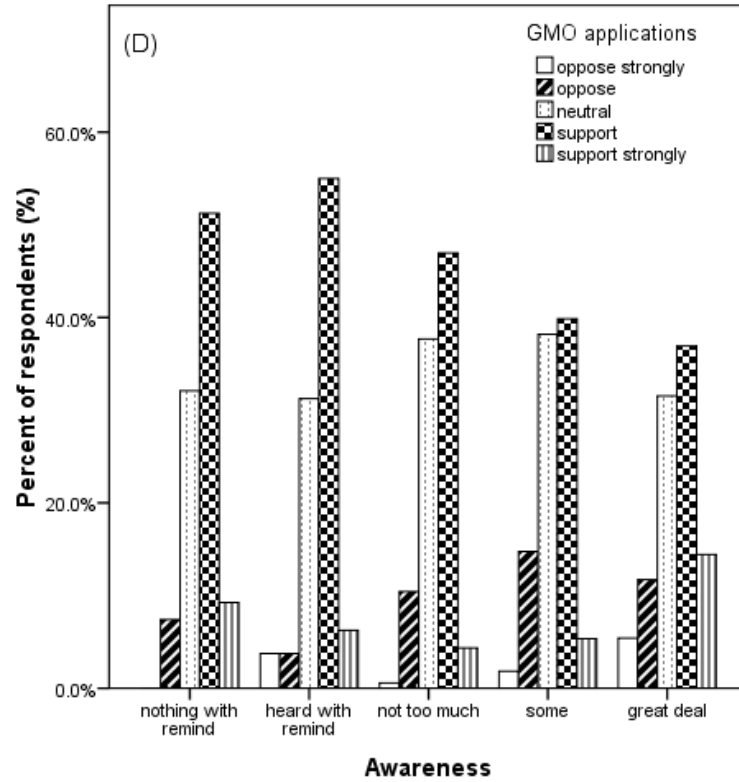
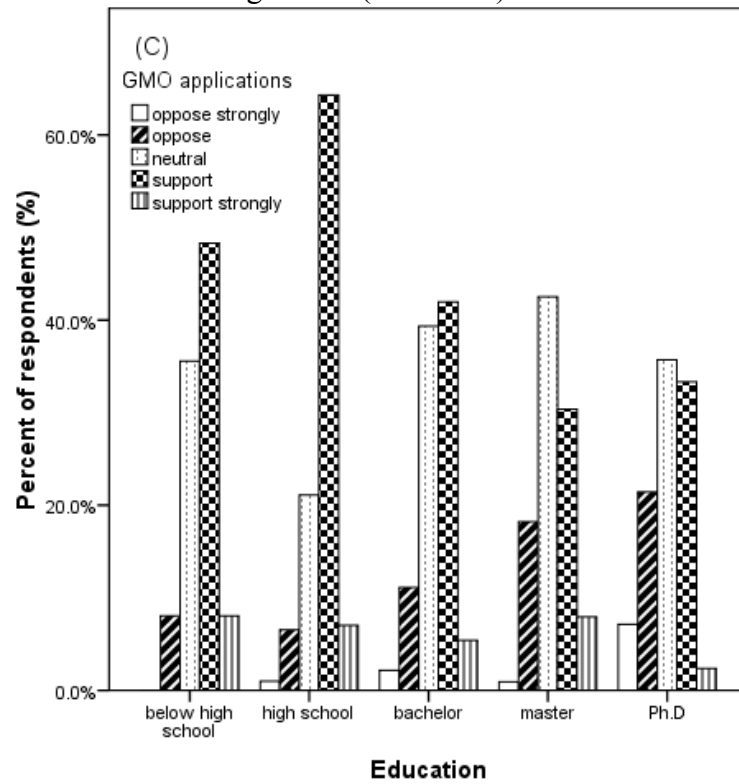
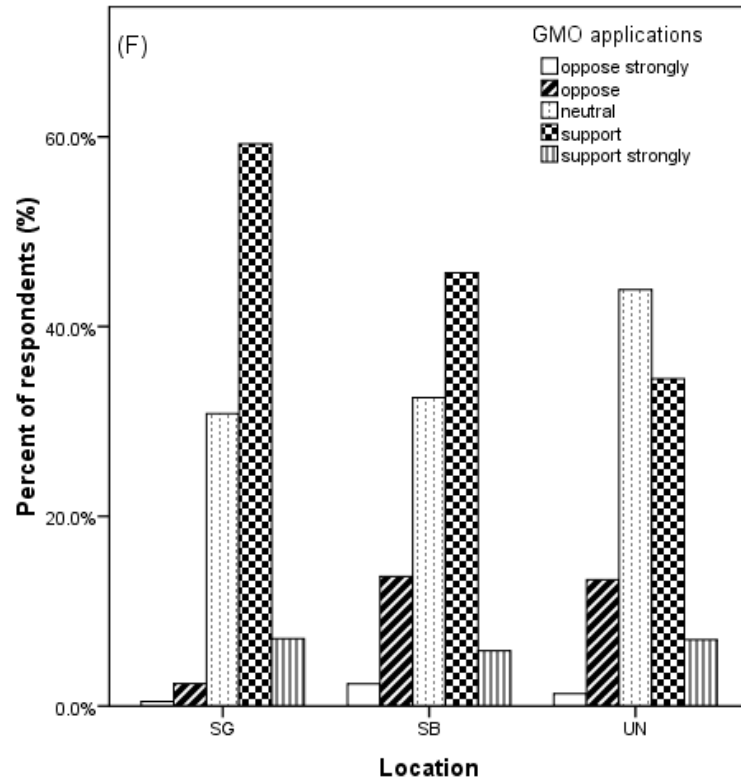
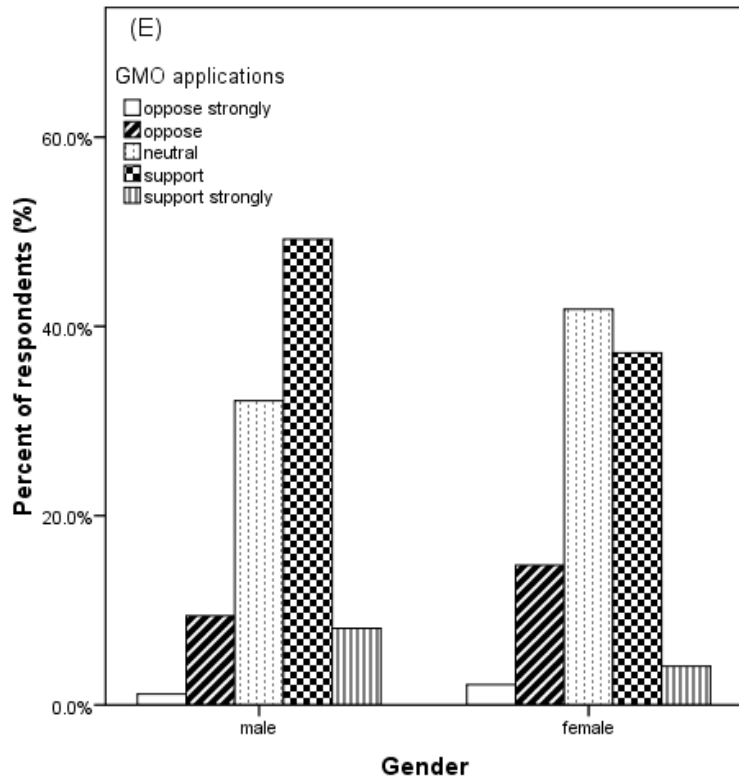


Figure 5.7 (continued)



#### **5.3.1.4 IR rice**

##### **5.3.1.4.1 AWARENESS and ACCEPTANCE of the Commercial Release of IR Rice**

Of all respondents surveyed, 60.2% had not heard that the “Chinese government is considering approving the commercial release of transgenic rice” before this survey or were not sure, while the rest had heard about this more or less. The percentage of respondents who had heard of this possibility in SG, SB AND UN were 33.1, 41.3 and 44.5%, respectively. For an undecided and unreleased policy, this AWARENESS level was high. This could be attributed to the debates reported in the Chinese media, including the ‘Rice is Life’ campaign undertaken by Greenpeace (Keeley, 2006).

The ATTITUDE structure for commercial release of IR rice was similar to that for GMO APPLICATIONS. Many respondents (38.9%) supported the commercial release of IR rice, strongly or not, while opponents accounted for 14.3%. A large proportion (30.3%) held a neutral position and 16.4% were not sure. Supporters in the UN, SB and SG test groups were 34.2, 41.0 and 40.9%, respectively, while 20.7, 16.9, and 4.7% were opposed, respectively (Table 5.2).

A binary logistic regression model was applied to analyze the binary dependent variables, whether respondents were SURE or UNSURE about their position. Significant variables included AGE, EDUCATION, AWARENESS, NGO EXPERIENCE and LOCATION (Table 5.5). The probability of being UNSURE increased with increasing AGE (Figure 5.8 A). For those with higher EDUCATION or a higher AWARENESS level or NGO EXPERIENCE, the probability of being UNSURE was significantly lower (Figure 5.8 B, C, D). Compared to the UN samples, this probability for the SG samples was significantly higher, but there was no significant difference between the UN and SB survey groups (Table 5.2). For the subgroup of respondents who were SURE of their position, an ordinal logistic regression model was applied and significant variables included

EDUCATION, AWARENESS, NGO EXPERIENCE, GENDER and LOCATION (Table 5.5). Levels of EDUCATION and AWARENESS were inversely related to respondent's ACCEPTANCE, meaning that the probability of having a higher level of support for the commercial release of IR rice decreased with an increase in EDUCATION or AWARENESS levels (Figure 5.9 A, B). Respondents with NGO EXPERIENCE or males or had a significantly higher probability of being more supportive (Figure 5.9 C). Compared to the UN samples, the SG samples had a significantly higher probability of being supportive while the SB samples did not (Figure 5.9 D).

It was surprising that respondents with environmental NGO EXPERIENCE had a significantly higher probability of being more supportive of the commercial release of

Table 5.5 Parameter estimates for ACCEPTANCE of commercial release of IR rice. The reference category is: SURE

<i>X</i> Explanatory Variables	Binary logistic regression model <sup>a</sup> : sure or not sure			Ordinal logistic regression for respondents who were sure their position		
	Estimate $\beta$	Std. Error	Sig.	Estimate $\beta$	Std. Error	Sig.
AGE	0.015	0.008	0.045	-0.007	0.006	0.294
EDUCATION	-0.347	0.102	0.001	-0.305	0.069	0.000
AWARENESS	-0.352	0.071	0.000	-0.140	0.052	0.008
NGO EXPERIENCE: -/+	0.840	0.324	0.009	-0.391	0.172	0.023
GENDER: MALE/FEMALE	-0.163	0.154	0.291	0.428	0.107	0.000
BIOLOGY BACKGROUND: -/+	0.055	0.368	0.882	0.091	0.164	0.579
LOCATION: SB VS. UN	-0.095	0.269	0.724	0.062	0.139	0.654
SG VS. UN	0.742	0.297	0.012	0.742	0.197	0.000

Figure 5.8 ACCEPTANCE of the commercial planting of IR rice. ACCEPTANCE with (A) AGE; (B) EDUCATION; (C) AWARENESS; (D) with or without environmental NGO EXPERIENCE.

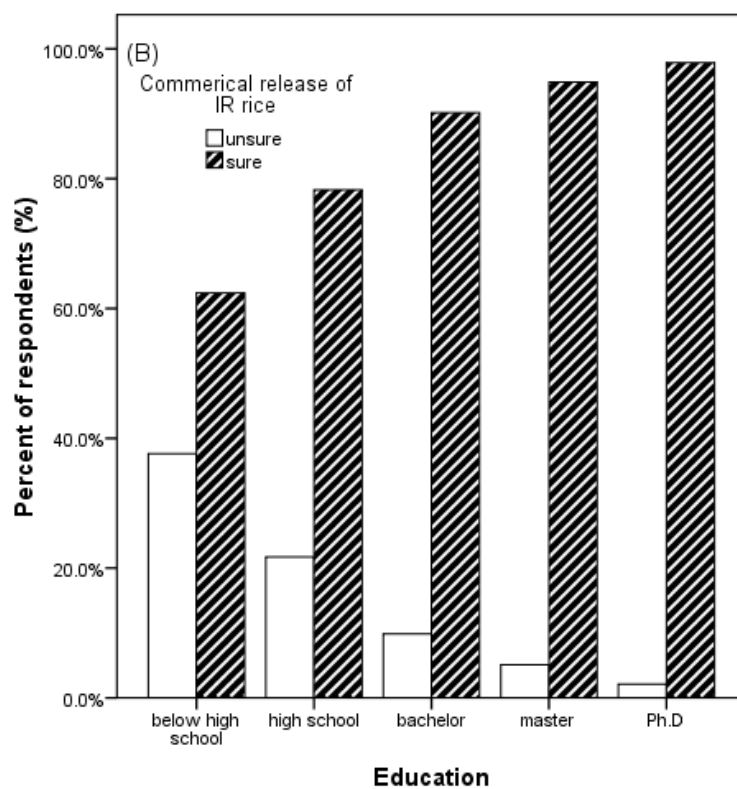
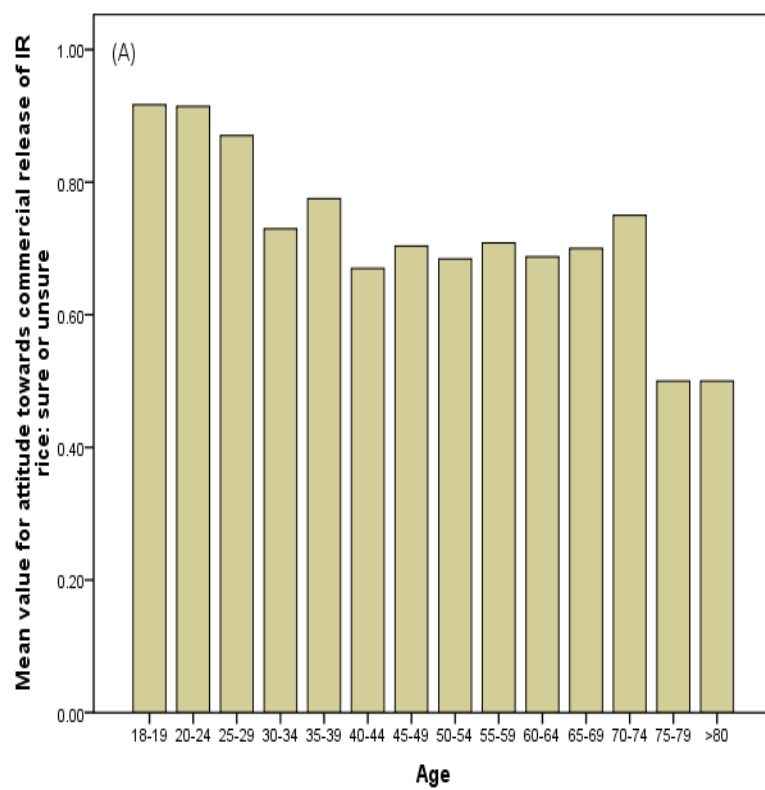
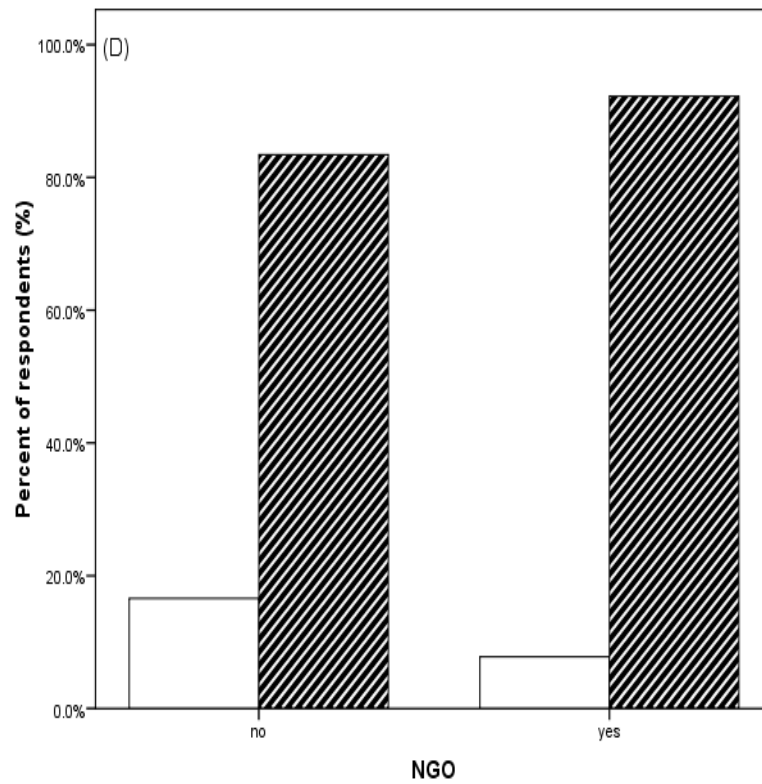
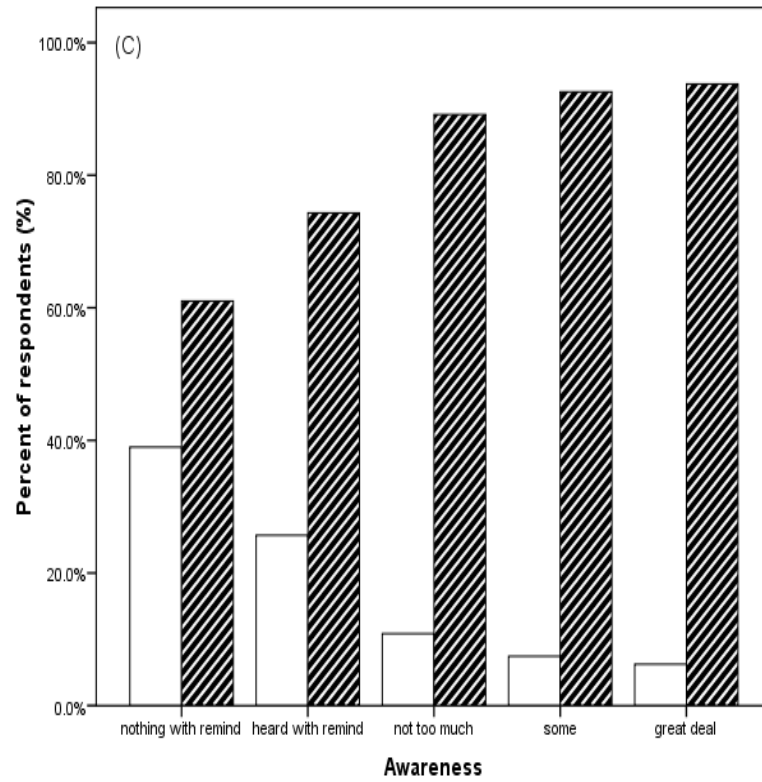


Figure 5.8

Figure 5.8 (continued)





IR rice. Some environmental NGOs are famous for their opposition to GMOs, such as Greenpeace. However, diversity exists in the environmental NGO world as well. This was consistent with my interview results, which showed that respondents in different environmental NGOs had different AWARENESS levels and ATTITUDES towards GMOs.

#### **5.3.1.4.2 WTP or WTA for IR Rice**

Benefits and potential risks of IR rice were provided to those surveyed in a random order. Clearly, different information led to different results. If the price of IR rice was the same as traditional rice, 63.4% said that they would buy IR rice when positive effects of IR rice on the environment were communicated to them and 60.5% would buy IR rice after knowing that no case has been reported where IR rice has had a negative effect on human health. On the contrary, after knowing about potential risks for the environment and human health, only 35.8% and 30.9% said that they would buy IR rice. Consistently, Ho and Vermeer (2004) also found the percentage of respondents who would buy GM foods dropped sharply from 78% to 52% when they were provided with information of the benefits and then the potential risks. These results indicate that respondents' reactions change in response to different kinds of information or different viewpoints. However, as Rowe (2004) pointed out, "the public acceptance of GM foods is much more complex than simply communicating their benefits".

If the price of IR rice was different from traditional rice, 44.6 - 47.4% of the respondents stated that they would buy IR rice with either a lower or a higher price, regardless of how the benefits or risks were communicated (Table 5.6). For respondents who stated their WTP or WTA, the average values were quite similar in all cases, about 16.1% WTP and 34.6% WTA (Table 5.7). If positive effects of IR rice on environment and human health were communicated, 18.4 - 19.1% of respondents

would not buy IR rice, even if a discount was provided, and 35.0 - 35.6% of respondents were not sure; while these two percentages changed to 32.0 - 35.7% and 19.8 - 20.7%, respectively, with information provided about the potential risks. These results indicated that positively stated information led to more UNSURE respondents, while negatively stated information caused more extremely strong opponents.

Table 5.6 Percentage of respondents whose ATTITUDE towards IR rice changed after either the benefits or the risks of GM crops was provided to them.

Information provided	Percentage of respondents (%)				
	Will buy			Will not buy in any case	Not sure
	WTP + WTA	Will buy providing negative information under same price condition	Total		
Positive: environment	46.6	-	46.6	18.4	35.0
Positive: human health	47.3	-	47.3	19.1	35.6
Negative: environment	13.6	31.0	44.6	35.7	19.8
Negative: human health	11.6	35.8	47.4	32.0	20.7
Overall	36.0	-	36.0	28.6	35.4

Table 5.7 Average WTP or WTA after providing different information.

Information	WTP (%)	WTA (%)
Positive: environment	15.5	36.3
Positive: human health	16.2	36.7
Negative: environment	-	36.8
Negative: human health	-	37.8
Overall	16.1	34.6

Note: WTP was not asked when only potential risks were communicated.

Figure 5.9 ACCEPTANCE of the commercial planting of IR rice for the subgroup of respondents who were SURE of their position. ACCEPTANCE with (A) EDUCATION; (B) AWARENESS; (C) with or without environmental NGO EXPERIENCE; (D) GENDER.

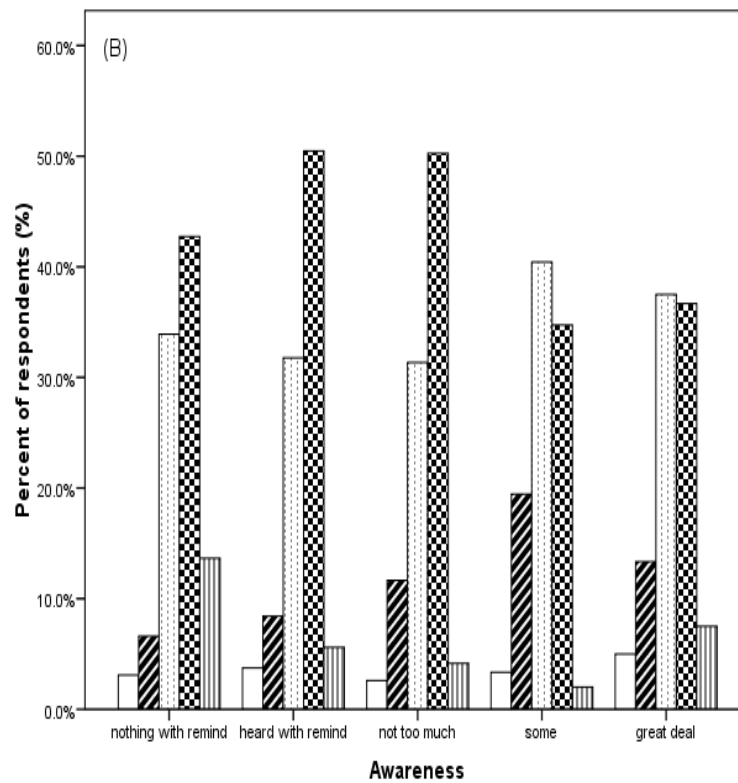
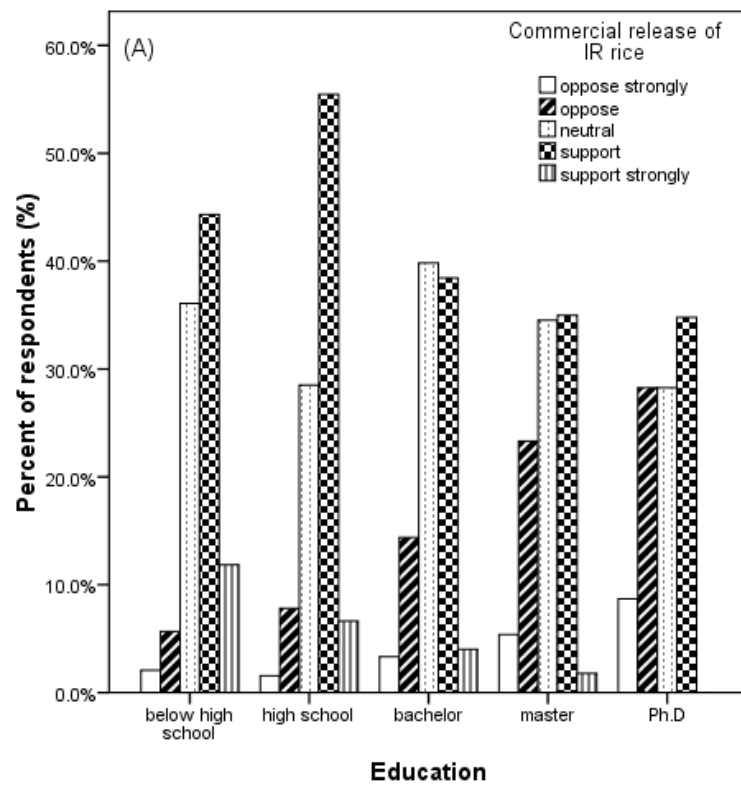


Figure 5.9

Figure 5.9 (continued)

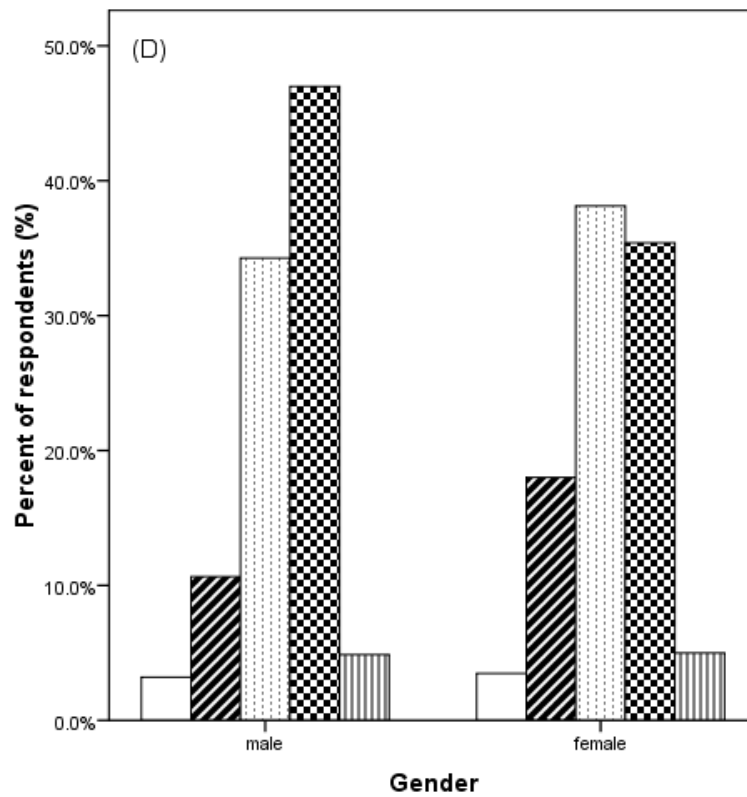
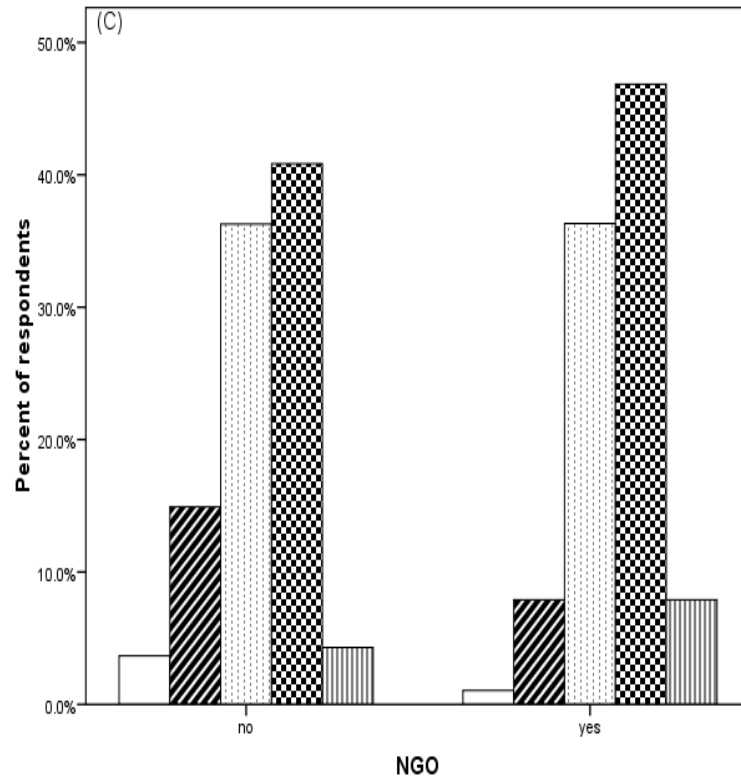
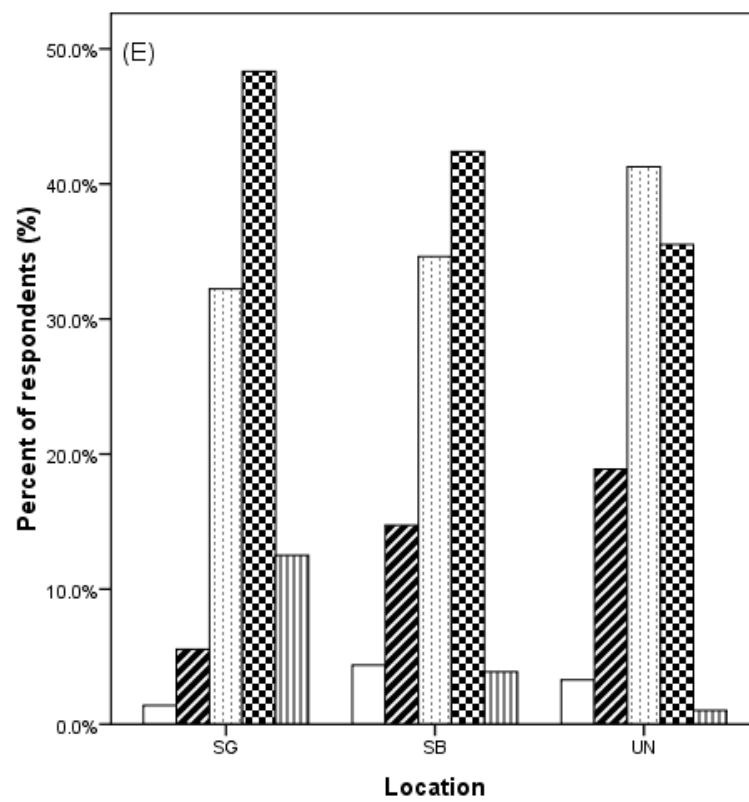


Figure 5.9 (continued)



Consumer purchasing behavior in the market is based on all kinds of information that has been received, rather than any single factor. After receiving all kinds of information, if the price of IR rice was same as the traditional rice, 56.2% of respondents stated that they would not buy IR rice, higher than when responding to negative information (30.9-36.8%), but lower than when responding to positive information (63.4-60.5%). Under the same price condition, significant variables ( $p < 0.05$ ) in the binary logistic regression model were environmental NGO EXPERIENCE, GENDER, LOCATION and PREVIOUS ATTITUDE towards commercial release of IR rice in the Wald Test (Table 5.8). In this model, only four statements were included for respondents' previous ATTITUDE (support, neutral, oppose or unsure), combining "strongly" or not strongly into one category.

Table 5.8 Parameter estimates for WTP/WTa for IR rice after receiving information about the benefits or risks of GM crops.

<i>X</i> Explanatory Variables	Binary logistic regression model <sup>a</sup> : Buy or not buy if the price of GM IR rice was the same as the traditional rice		
	Estimate $\beta$	Std. Error	Sig.
AGE	0.009	0.006	0.169
EDUCATION	0.081	0.076	0.281
AWARENESS	-0.014	0.056	0.796
NGO EXPERIENCE: -/+	0.492	0.187	0.009
GENDER: MALE/FEMALE	-0.267	0.114	0.020
BIOLOGY BACKGROUND: -/+	0.151	0.191	0.428
LOCATION: SB VS. UN	-0.344	0.157	0.029
SG VS. UN	-0.197	0.209	0.347
PREVIOUS ATTITUDE = unsure vs. support	1.126	0.169	0.000
oppose vs. support	2.142	0.210	0.000
neutral vs. support	1.033	0.133	0.000

a. The reference category is: buy

The probability of respondents with environmental NGO EXPERIENCE or MALES to buy IR rice was significantly higher than for respondents without environmental NGO EXPERIENCE or FEMALES (Figure 5.10 A, B). Compared to UN respondents, the SB respondents had a significantly higher ( $p=0.03$ ) probability of buying IR rice (Figure 5.10 C). The probability of supporters of the commercial release of IR rice to buy IR rice was significantly higher ( $p<0.001$ ) than other respondents (Figure 5.10 D). The 85.1% of respondents who opposed and the 87.2% of respondents who opposed the commercial release of IR rice strongly would not buy IR rice. For respondents who claimed that they supported GMO RESEARCH and supported strongly the commercial release of IR rice, 65.9% and 47.9% would buy IR rice, respectively. More than half of the neutral and unsure respondents, 61.8% and 68.4%, respectively, would not buy IR rice. These results indicate that opponents were more insistent on their position than supporters were and neutral or unsure respondents were more likely to be negatively influenced than to be supportive given more information.

If the price of IR rice was different from traditional rice, after considering symmetrical information about TCs, fewer respondents would buy IR rice (36.0%). Of these, 9% were WILLING TO ACCEPT IR rice if the price were discounted and 27% were WILLING TO PAY even more. The percentage of unsure respondents was 35.4%. The percentage of respondents who would not buy IR rice in any case (28.6%) was lower than when provided with negative information, but higher than when they were provided with positive information. Significant variables in the multinomial logistic regression model were GENDER, LOCATION and PREVIOUS ATTITUDE towards commercial release of IR rice (Table 5.9). Compared to MALES, the probability that FEMALES would not buy IR rice in any case was significantly higher than those who were WILLING TO PAY or WILLING TO ACCEPT it (Figure 5.11A). Compared to the



Figure 5.10 “If the price of IR rice was same as the traditional rice, will you buy or not buy IR rice after considering all information provided”. WILLINGNESS (A) with or without environmental NGO EXPERIENCE; (B) with GENDER; (C) in different LOCATIONS; (D) with PREVIOUS ATTITUDE toward commercial release of IR rice.

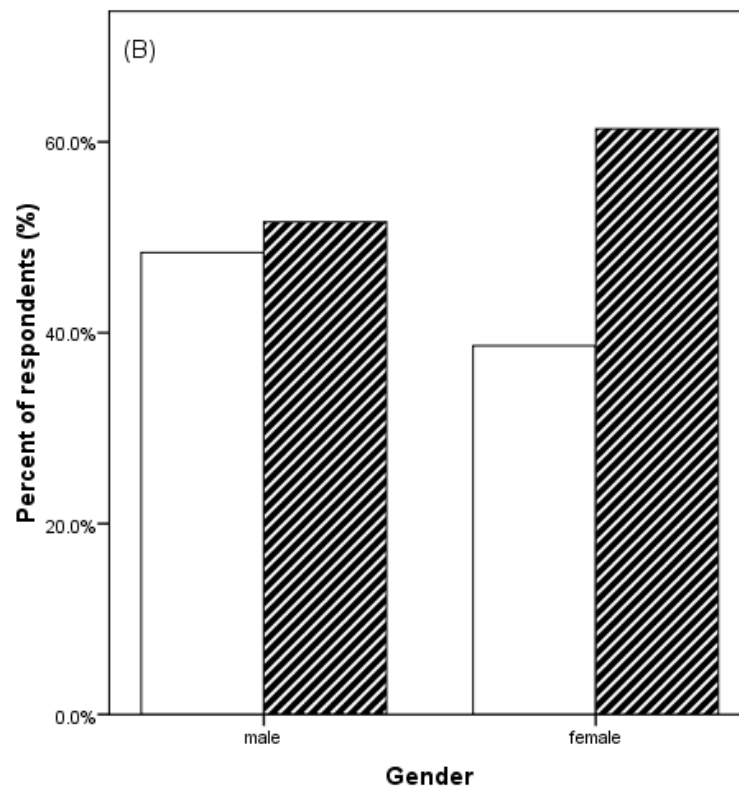
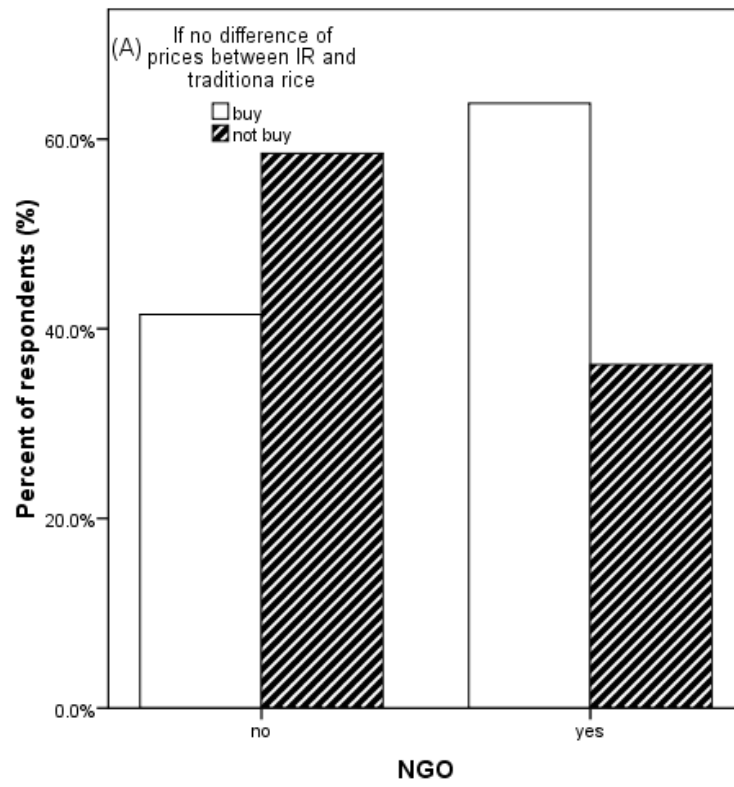
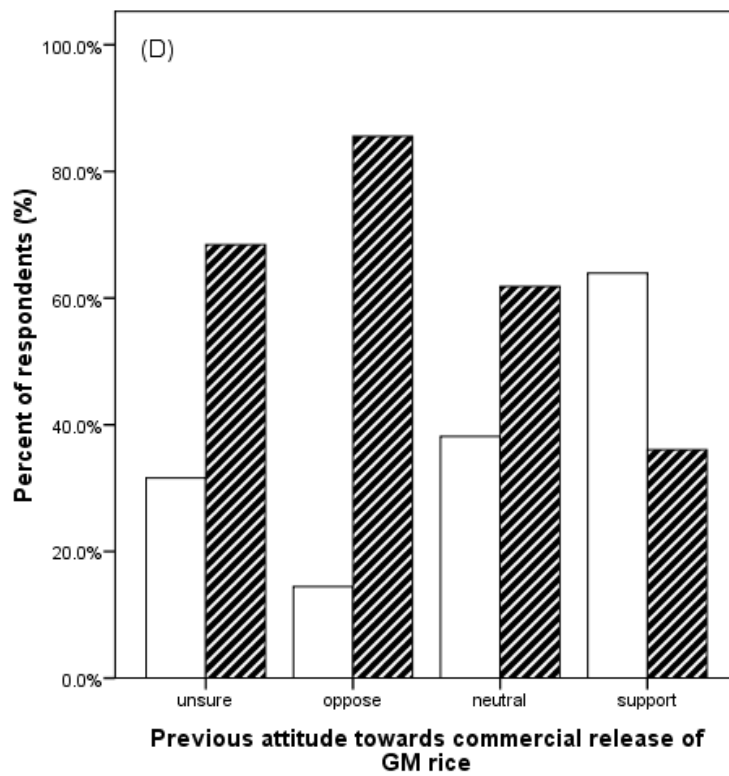
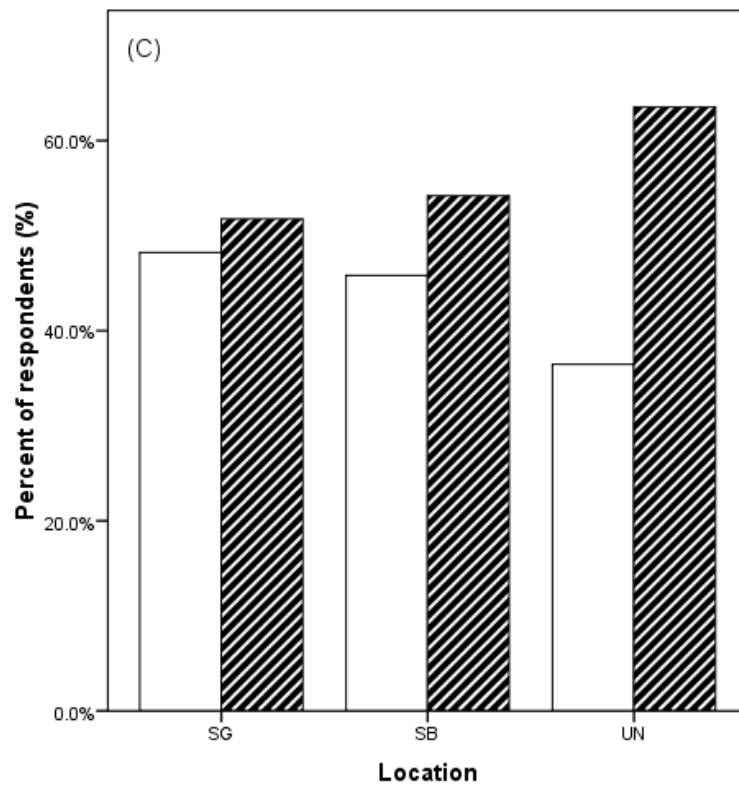


Figure 5.10

Figure 5.10 (continued)



UN respondents, the probability of the SB or SG respondents not to buy or be unsure of buying was significantly higher ( $p < 0.05$ ) than those who were WILLING TO PAY for or WILLING TO ACCEPT it (Figure 5.11 B). Respondents' PREVIOUS ATTITUDE towards the commercial release of IR rice was also a significant factor (Figure 5.10 C). Compared to the probability of being WILLING TO PAY for or WILLING TO ACCEPT IR rice, the probability of not buying it was in the order of their PREVIOUS ATTITUDE: oppose > unsure > neutral > support, while the probability of being unsure was in the order of unsure > neutral > oppose > support, although the difference between those opposing and supporting was not significant.

For respondents who clarified their WTP and WTA, LOCATION and PREVIOUS ATTITUDE towards commercial release of IR rice were significant variables ( $p < 0.05$ ) in the Wald Test (Table 5.10). In the ordinal logistic regression model, WTA was treated as a negative value. The orders of the probability of having a higher willingness to buy IR rice were SB>SG>UN regarding LOCATION and support>unsure>neutral>oppose with regard to PREVIOUS ATTITUDE (Figure 5.11 A, B). The significant effect of PREVIOUS ATTITUDE indicated that the effectiveness of advertising the benefits of GM products may be limited if consumers have already developed their ATTITUDE.

#### **5.3.1.4.3 GM Rice with Higher Nutrition**

Respondents had a more positive ATTITUDE towards GM rice if it also had a higher nutrient content, which is a combination of characteristics that is not being considered for commercial release at this time. A total of 70.3% would buy nutrient augmented rice at the same price as traditional rice. These percentages in the SG, SB and UN test groups were 68.5%, 75.5%, and 65.1%, respectively. Moreover, 44.4% of those surveyed would pay more for GM rice with higher nutrients. Li *et al.* (2003) found an

Table 5.9 Likelihood Ratio Tests for respondents' ATTITUDE toward IR rice using a multinomial logistic regression model after receiving symmetrical information if the price of GM IR rice were different from that of traditional rice.

$X$ Explanatory Variables	Model Fitting Criteria	Likelihood Ratio Tests		
	-2 Log Likelihood of Reduced Model	Chi-Square	df	Signif.
Intercept	2.753E3 <sup>a</sup>	0.000	0	.
AGE	2.758E3	5.252	2	0.072
EDUCATION	2.757E3	3.532	2	0.171
AWARENESS	2.756E3	2.961	2	0.228
NGO EXPERIENCE	2.754E3	0.968	2	0.616
GENDER	2.759E3	6.461	2	0.040
BIOLOGY BACKGROUND	2.755E3	1.877	2	0.391
LOCATION	2.774E3	21.110	4	0.000
PREVIOUS ATTITUDE	2.902E3	149.414	6	0.000

Table 5.10 Parameter estimates for WTP/WTa for GM IR rice after receiving all kinds of information.

$X$ Explanatory Variables	Ordinal logistic regression for respondents who clarified clearly WTP and WTA		
	Estimate $\beta$	Std. Error	Signif.
AGE	-0.012	0.009	0.204
EDUCATION	-0.199	0.105	0.059
AWARENESS	0.069	0.075	0.356
NGO EXPERIENCE: -/+	-0.459	0.256	0.072
GENDER: MALE/FEMALE	0.283	0.152	0.063
BIOLOGY BACKGROUND: -/+	-0.050	0.232	0.829
LOCATION: SB VS. UN	1.121	0.205	0.000
SG VS. UN	0.540	0.271	0.046
PREVIOUS ATTITUDE = unsure vs. support	-0.611	0.261	0.019
oppose vs. support	-1.295	0.264	0.000
neutral vs. support	-0.913	0.183	0.000

Figure 5.11 If the price of IR rice was different from traditional rice, respondent's WILLINGNESS TO PAY for IR rice after considering symmetrical information provided. WILLINGNESS (A) vs. GENDER; (B) in different LOCATIONS; (C) with PREVIOUS ATTITUDE toward commercial release of IR rice.

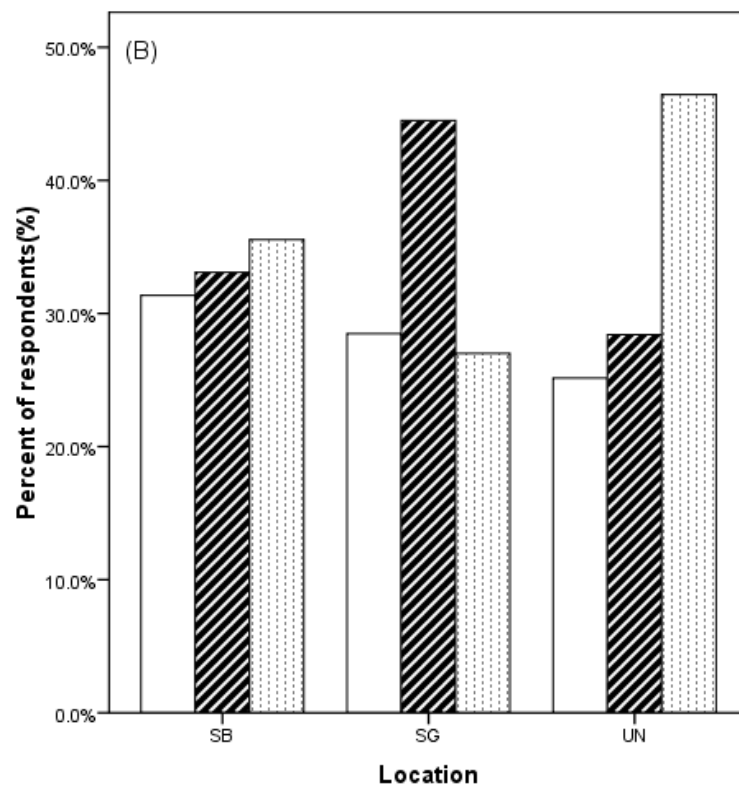
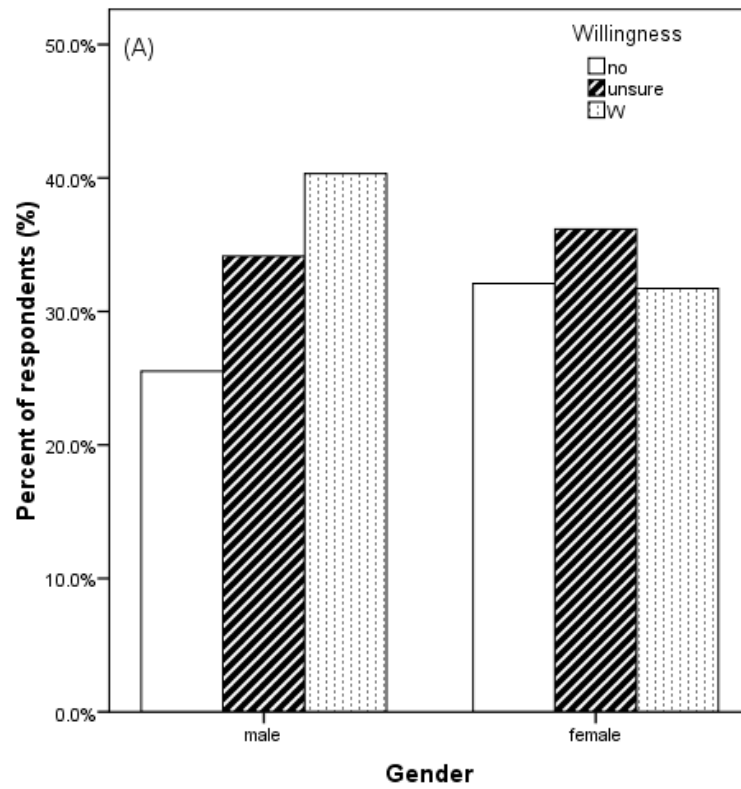
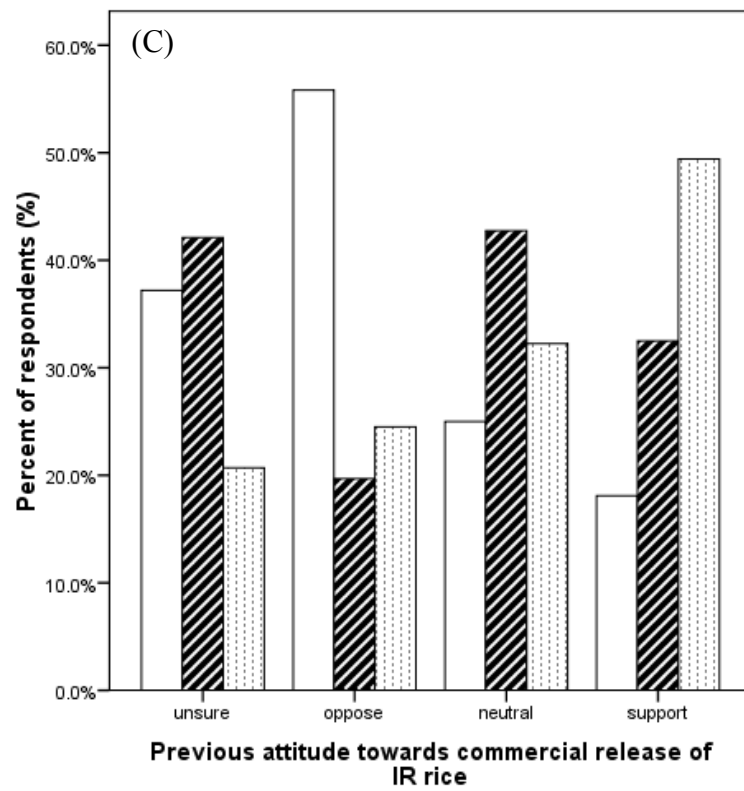


Figure 5.11

Figure 5.11 (continued)





even more positive attitude toward GM rice with additional vitamins, 80% of respondents in China expressed their WILLINGNESS TO BUY when nutrient enriched rice was offered for the same price. Zhong *et al.* (2002) reported that 46% of the respondents would buy GM food with improved nutrients, 15% higher than food from IR crops. These results indicated consumers surveyed were more interested in GM products that could bring a direct benefit to them.

### **5.3.2 Interviews with Focus Groups**

The 37 farmers in Beijing and 30 farmers in Guanyun, Jiangsu Province, were interviewed to assess their AWARENESS and understanding of TCs. Four government officers, five NGO members, three policy scientists and 18 scientists in biology or environmental science were interviewed as well.

#### **5.3.2.1 Farmers**

Only three of 37 farmers in Beijing had heard of TCs before this interview. Their source of information was television in all cases. In Guanyun, 20 farmers were from the area where IR cotton had been planted, seven persons mentioned that they had heard of TCs or IR cotton before. However, no one knew the mechanism by which IR rice became insecticidal.

IR rice was not attractive to farmers in this study. Two major concerns of farmers were IR rice's cost and its insect resistance effectiveness. Both in Beijing and Jiangsu Province, the cost of pesticides and seeds accounted for only a small part of the total amount of inputs for agriculture, far less than that for fertilizers (P and N) or field management practices, such as tillage and harvesting. Hence, farmers were more eager to have GM crops that use less fertilizer. Some farmers who had or were planting rice worried about the effectiveness of IR rice. Based on their past experiences, pests in

rice fields varied in different locations and in different years. They were not interested in buying seeds that protect rice from only a few pests. Moreover, they felt that yield losses due to rice diseases were more serious than those from insect pests.

#### **5.3.2.2 Government Officers**

Most of the government officers refused to accept an interview. This may be because they were cautious and afraid of expressing a different opinion from that of the government. One officer claimed that the policy for GMOs should be based on the national benefit and then stopped the discussion. Another officer from the same department did not express any opinion during the interview.

#### **5.3.2.3 NGOs**

Respondents from different NGOs held quite different opinions. Their AWARENESS and knowledge levels varied as well. One person objected to all kinds of GMO APPLICATIONS, however, another supported GMOs for bio-energy, but not for food. An interesting point proposed by a person from an NGO advocating for animal welfare was that human beings should restrain their demand for natural resources. This person believed that this was the solution for all current problems.

#### **5.3.2.4 Scientists**

In the area of policy science, some respondents were concerned about who would reap the benefits and who would assume the risks of GMOs. One person worried that farmers lacked a channel to express their concerns or claim their rights in China's current political system and would assume all the risks without getting any benefits. The lack of farmers' AWARENESS and knowledge of GMOs was another concern, such that scientists thought farmers did not have the ability to make proper choices. Biological and environmental scientists were more concerned about human health and

environmental effects than policy scientists. Respondents with biological science backgrounds tended to be more supportive of GMOs.

### **5.3.3 Future Scenarios**

Although the percentage of opponents to GMO APPLICATIONS or the commercial release of IR rice was quite low, a large proportion of neutral or unsure respondents were observed and they were more likely to be opponents than to be supporters. Moreover, based on interview results, IR rice seeds with protection from a limited number of pests would not be attractive to farmers, the users of GM seeds. These results indicated a possible resistance to the commercial release of IR rice. This was consistent with Ho and Vermeer (2004), who predicated future scenarios wherein Chinese consumers resist accepting transgenic food.

With economic development, people's EDUCATION level (which was positively related to AWARENESS) will increase in China. Moreover, younger respondents had a higher AWARENESS level. So, AWARENESS in the future may increase in China. However, the future ACCEPTANCE level might still be lower as there was an inverse relationship between AWARENESS and ACCEPTANCE in the survey dataset.

## **5.4 Conclusions**

The AWARENESS level of consumers was low in Guanyun, but relatively high in Beijing, and extremely high for universities students. Beyond professed AWARENESS, respondent's actual knowledge was still quite limited. Respondents had a higher ACCEPTANCE level of GMO RESEARCH than for GMO APPLICATIONS. The percentage of opponents to GMO RESEARCH or GMO APPLICATIONS was quite low. A large proportion of respondents held neutral positions or were not sure. Opponents were more insistent in their position than supporters were, but those who were holding a neutral position

were more likely to become more negative than positive when given additional information.

Respondents' ATTITUDES towards IR rice were quite different depending on whether the potential benefits or risks were communicated to them. Information restricted to the benefits of TCs led to more unsure respondents, while information restricted to the risks of TCs yielded stronger opponents. Farmers were more interested in GM rice with higher nutrient contents or rice that is more responsive to fertilizer than they were in IR rice.

With a higher level of EDUCATION, the probability of having a higher AWARENESS or lower level of support for GMO APPLICATIONS was significantly higher. Compared to FEMALES, MALES had a significantly higher probability to be SURE or more supportive of GMO APPLICATIONS. With environmental NGO EXPERIENCE, the probability of having a higher AWARENESS or being SURE of their position for GMO APPLICATIONS was significantly higher. With a BIOLOGY BACKGROUND, the probability of having a higher AWARENESS or of being more supportive of GMO APPLICATIONS was significantly higher. With a higher AWARENESS level, the probability of being UNSURE or more supportive of GMO APPLICATIONS or the commercial release of IR rice was significantly lower. Moreover, after providing both the benefits and potential risks, people's PREVIOUS ATTITUDE towards the commercial release of IR rice was still a significant factor affecting people's WILLINGNESS TO PAY and WILLINGNESS TO ACCEPT TCs. Supporters were more likely to buy IR rice and opponents were more unlikely to buy it.

IR rice may not be attractive to farmers as they were concerned about the cost and effectiveness of seeds. Farmers surveyed in this study were more interested in GM rice with improved fertilizer use efficiency than IR rice.

This study provides valuable information about Chinese consumer's perceptions,

which should be considered in the policy-making process and is important for the successful deployment (or commercial release) of GM products. Consumer choice behavior after receiving symmetrical information should help the industry to develop and improve their marketing strategies. My results indicated that the commercial release of IR rice may encounter consumer resistance. Predictions for future scenarios suggest that the AWARENESS is likely to increase with time but that ACCEPTANCE might decrease as indicated by predictive model output.

Government regulatory bodies should be very careful when establishing policies for the commercial release of IR rice with regard to the possible resistance from consumers. The needs of farmers should be taken into consideration fully. The balance between the perceived benefits and the potential risks also needs to be considered. If the benefits are as limited as farmers indicated they thought they were, then IR rice seeds would not be attractive to them. In this case, the potential risks become more meaningful and they may choose not to consume them. Focusing on transgenic rice with improved fertilizer use efficiency may be a better option for addressing farmer's needs.

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## APPENDIX A Decomposition of litterbags for Chapter 2

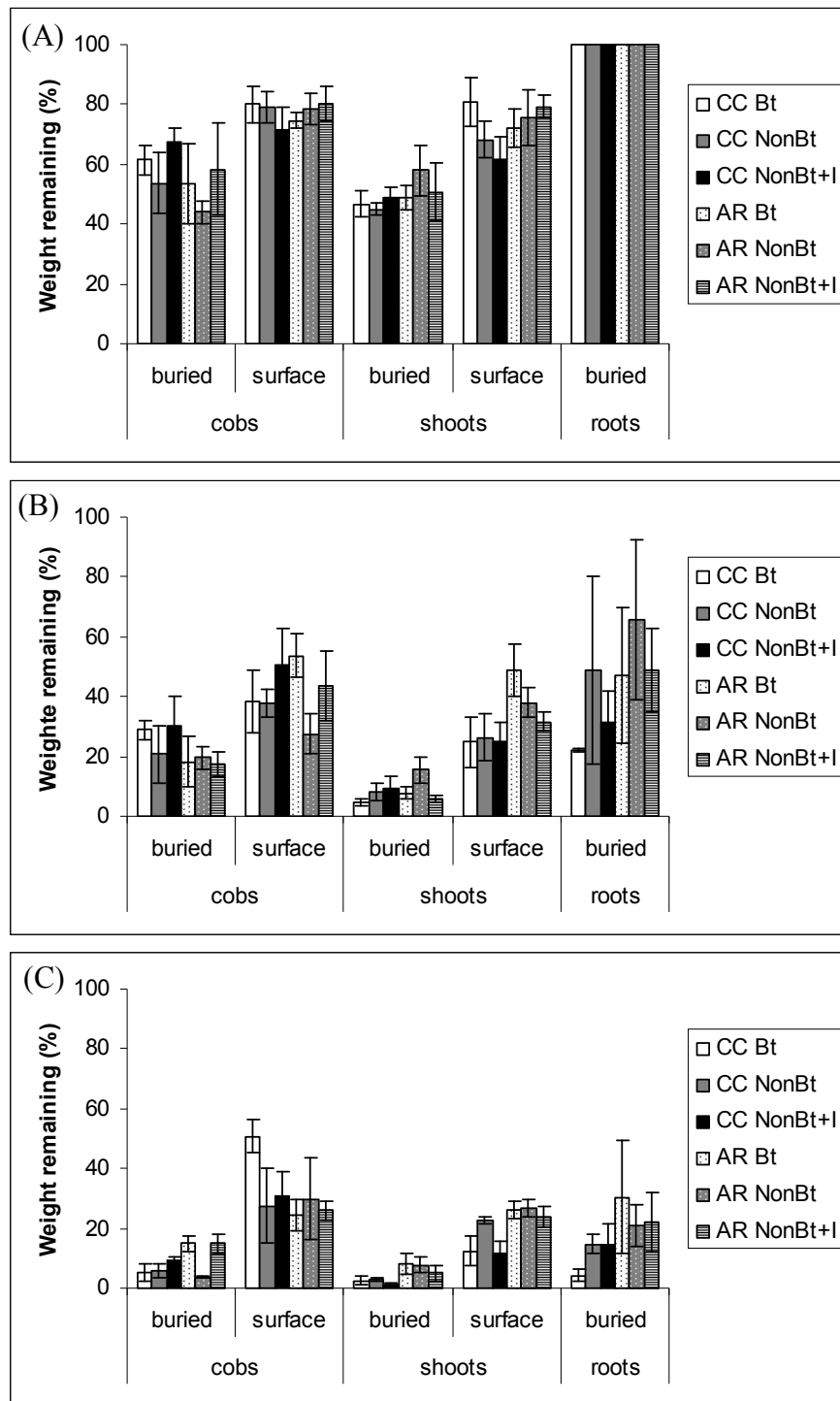


Figure A. Decomposition of litterbags after (A) 3.5; (B) 15.5; (C) 25 months.



## APPENDIX B Questionnaire for Chapter 5

### Part 1

**S1** How much have you heard about genetically modified organisms (GMOs) before this survey?

Great deal	Go to S3
Some	Go to S3
Not too much	Go to S3
Noting at all	Go to S2
Don't know	Go to S2

**S2** In order to obtain particular desirable traits, one or several genes associated with the traits are transferred from one organism to another, creating a GMO. For example, the transfer of bacterial genes that can be expressed as an insecticide to plants can make the plant be resistant to certain pest. After reading this description, can you remember whether you have heard anything about GMOs before?

Yes	Go to S3
No	Go to S6

**S3** How much do you think you know about GMOs?

Great deal	Some	Not too much	Nothing at all	Not sure
1	2	3	4	5

**S5** Please indicate where you obtained information about GMOs. Generally, was this information in favor, oppose or neutral?

	Strongly oppose	Oppose	Neutral	Favor	Strongly Favor
Media: newspaper	1	2	3	4	5
Media: TV	1	2	3	4	5
Media: Internet	1	2	3	4	5
People you know	1	2	3	4	5
Literatures	1	2	3	4	5
Other, please specify	1	2	3	4	5

**S6** Generally, do you favor or oppose the development of genetically modified foods by scientist and their application in agriculture or industry? If you haven't heard about GMOs before this survey, please choose a position based on the description in question S2.

	Strongly oppose	Oppose	Neutral	Favor	Strongly Favor	Unsure
Development:	1	2	3	4	5	0
Application:	1	2	3	4	5	0

**S7** Do you think genetically modified foods are basically safe?

Strongly oppose	Oppose	Neutral	Favor	Strongly Favor	Unsure
1	2	3	4	5	0

## Part 2

**S1** Do you favor or oppose mandatory labeling of genetically modified (GM) food?

Strongly oppose	Oppose	Neutral	Favor	Strongly Favor	Unsure
1	2	3	4	5	0

**S2** Do you know the labeling of GM food is mandatory in China?

Yes      No

**S3** How much have you noticed the label of food in market containing GM ingredients?

Great deal	Some	Not too much	Nothing at all	Unsure
1	2	3	4	5

**S4** How much have you heard that China is considering approving the commercial planting of insect-resistant (IR) rice, one staple food that you may eat everyday?

Great deal	Some	Not too much	Nothing at all	Unsure
1	2	3	4	5

**S5** Where did you hear about that (S4)? Generally, was this information in favor, oppose or neutral?

	Strongly oppose	Oppose	Neutral	Favor	Strongly Favor
Media: newspaper	1	2	3	4	5
Media: TV	1	2	3	4	5
Media: Internet	1	2	3	4	5
People you know	1	2	3	4	5
Literatures	1	2	3	4	5
Other, please specify	1	2	3	4	5

**S6** Generally, do you favor or oppose the approval of the commercial planting of IR rice, one staple food that you may eat everyday?

Strongly oppose	Oppose	Neutral	Favor	Strongly Favor	Unsure
1	2	3	4	5	0

**S7** If the IR rice can benefit environment through reducing the spraying of insecticide, will you buy it when its price is the same as non-GM rice?

Yes    No

If you answer yes, when the price is different from traditional rice, will you consider paying a premium for IR rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy    Unsure

If you answer no, when the price is different from traditional rice, will you consider to purchase IR rice after getting a discount compared to non-GM rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy    Unsure

**S8** After knowing the history of GM foods is too short to fully evaluate their potential

effect on human health, as someone argue, though so far there is no evidence showing GMOs poses risk to human beings, will you buy it when the price of IR rice is the same as non-GM rice?

Yes      No

If you answer no, when the price is different from traditional rice, will you consider to purchase IR rice after getting a discount compared to non-GM rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy      Unsure

**S9** After knowing GMOs may have potential effects on environment, such as gene flow, that means it is likely that transgenes will move to wild rice, will you buy it when the price of IR rice is the same as non-GM rice?

Yes      No

If you answer no, when the price is different from traditional rice, will you consider to purchase IR rice after getting a discount compared to non-GM rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy      Unsure

**S10** Considering all the above various perspectives (including reducing the spray of insecticide, potential effects on human health, and gene flow between IR plants and wild species), will you buy IR rice when the price of IR rice is the same as non-GM rice?

Yes      No

If you answer yes, when the price is different from traditional rice, will you consider paying a premium for IR rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy      Unsure

If you answer no, when the price is different from traditional rice, will you consider to purchase IR rice after getting a discount compared to non-GM rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy      Unsure

**S11** If the GM rice has improved nutrient, will you buy it when the price of pest-resistant GM rice is the same as non-GM rice?

Yes    No

If you answer yes, when the price is different from traditional rice, will you consider paying a premium for GM rice?

5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, Will not buy      Unsure

*(The order from S7 to S9 will be changed randomly)*

### **Part 3**

**S1** Do you or did you worked for environmental NGOs within five years?

Yes    No

**S2** Gender: Male Female

**S3** Age:

**S4** Please make your choice (circle one): Where is the Bt gene in Bt plants from?

Bacterial, Fungi, Animals, Other plants, don't know

**S5** What was the last level of education you completed and majors if applicable?

Less than high school graduate

High school graduate

Bachelor

Major:

Mater

Major:

Ph.D

Major:

**S6** Please specify how important the following options are in determining your attitude towards GMOs.

	How important?				
	Very	Somewhat	Not too much	Not at all	Unsure
The science involve	1	2	3	4	0
Your ethical beliefs	1	2	3	4	0
The impact it might have on you and your family	1	2	3	4	0
The impact it might have on environment	1	2	3	4	0
The trust you have in the people providing information	1	2	3	4	0
The trust you have in relevant government agencies	1	2	3	4	0

S7 How much you trust the information released from the following resources about GMOs?

	Great deal	Some	Not too much	Not at all	Unsure
Biological scientists	1	2	3	4	0
Environmental or ecological scientists	1	2	3	4	0
Government	1	2	3	4	0
Companies	1	2	3	4	0
Environmental NGOs, such as Greenpeace	1	2	3	4	0
Friends and family	1	2	3	4	0
Traditional news media, such as newspaper, TV	1	2	3	4	0
News media in internet- such as Sina.com	1	2	3	4	0
Internet-BBS	1	2	3	4	0

**S8** Income (RMB) per month in your house:

400	0 1	4000—4499	1 1
400—599	0 2	4500—4999	1 2
600—799	0 3	5000—5499	1 3
800—999	0 4	5500—5999	1 4
1000—1499	0 5	6000—6499	1 5
1500—1999	0 6	6500—6999	1 6
2000—2499	0 7	7000—7499	1 7
2500—2999	0 8	7500—7999	1 8
3000—3499	0 9	8000 or above	1 9
3500—3999	1 0	Refuse to answer	9 8